

MISTLETOE REPRODUCTIVE MUTUALISMS
IN A
WEST AFRICAN MONTANE FOREST

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Abstract

In this thesis I investigated the importance of plant-animal mutualisms to the reproductive success of three West African mistletoe species in two genera, *Globimetula braunii*, *Agelanthus brunneus* and *A. djurensis*, in Ngel Nyaki Forest Reserve, Nigeria.

The flowers of all three mistletoes were visited by 3 - 4 species of sunbird. *Agelanthus* flowers were also visited by honeybees (*Apis mellifera*) and a small social wasp species (Vespinae). *A. mellifera* appeared to be robbing nectar from the flowers of *A. brunneus*. To investigate the relative role of pollinators, I compared flower opening and fruit set amongst bagged, caged, natural, hand-selfed and hand-crossed treatments. The flowers of *G. braunii* were able to self-open on average 66% of the time when pollinators were excluded, whereas pollinators were essential to the flower opening mechanism of both *Agelanthus* spp. Insects were as effective at opening the flowers of *Agelanthus* spp. as sunbirds. However, flower opening ability did not translate directly into pollination effectiveness, as insect access alone did not result in significantly higher fruit set than that observed under the bagged condition. There was no significant evidence for autonomous selfing within any of the three mistletoes and thus reproduction was almost entirely reliant on 3 – 4 species of sunbird. Hand-pollinations of all three species indicated a high level of self-compatibility, and in one species, *G. braunii*, pollen limitation was evident (PLI = 0.504).

To investigate dispersal mutualisms amongst the three mistletoe species, fruit ripening and removal were monitored. The fruits of all three mistletoe species appeared to be removed rapidly from plants as they ripened, with few ripe or overripe fruits present on the branches at any time. Dispersal efficiency, or the total proportion of fruit crop removed across the fruiting season, was also very high (>90%) for the *Agelanthus* spp. but lower in *G. braunii*, for which almost a third of the total fruit crop was recorded undispersed in fruit nets beneath plants.

Mistletoes are an important component of West African montane forests. Disruption to mistletoe reproductive mutualisms may affect not only mistletoes and their mutualists directly, but also an entire network of species, all linked within a web of interactions. To protect these ecosystems from further degradation, increased community involvement and greater enforcement of laws set out to manage montane forest habitat across the region is essential. Without this support, the future of these ecosystems and the web of interacting species within remains tenuous.

Chapter 1: General Introduction

1.1 Introduction to Mutualisms

Interactions between plants and animals where both organisms mutually benefit from the exchange are ubiquitous throughout nature. Charles Darwin recognized both the complexity and importance of these interactions in his seminal work ‘Origin of Species’ - *“No one ought to feel surprise at much remaining as yet un-explained in regard to the origin of species and varieties, if he makes due allowance for our profound ignorance in regard to the mutual relations of all beings which live around us”* (Darwin 1859) p 68.

Despite 150 years of research into what Darwin coined “the entangled bank” of species and their interactions, there remains much to be learnt about these complex community networks and the mutualisms linking species therein.

The interaction between plants and their animal pollinators and dispersers epitomizes the value of mutualisms in maintaining ecosystem functioning. An estimated 98-99% of all angiosperms in tropical lowland forests are animal pollinated (Bawa 1990). Similarly, the vast majority of tropical woody plant species rely on vertebrates for the dispersal of their seeds (Jordano 1992).

It is important to recognise that despite the mutually beneficial outcome of these plant-pollinator and plant-disperser relationships, they are derived from evolutionary relationships that were originally antagonistic. That is, pollinators and dispersers are not adapted for these services; they are adapted to forage for food (e.g. pollen, nectar and fruits) and are exploiting the flowers and fruits of plants. Likewise, plants are exploiting their animal mutualists for the efficient transfer of gametes and seeds. This reciprocal exploitation is maintained through coevolution of traits which may lead to specialised reproductive mutualisms (Howe and Westley 1986, Thompson 1994). The degree of specialization, and hence the extent to which plant species have become reliant on their animal pollinators or dispersers remains largely unknown across innumerable ecosystems (Jordano 1987b, Johnson and Steiner 2000).

Both empirical and theoretical evidence suggest that generalisation is the rule rather than the exception in pollination systems (Waser et al. 1996), with each species interacting with a few to

several, or even hundreds of other species forming a complex network of mutually beneficial interactions (Jordano 1987b, Memmott 1999, Stanton 2003, Bascompte and Jordano 2007). Very few pollination interactions are entirely obligate, with highly specialised species-specific mutualisms such as those found in the tropics between the yucca and yucca moth (Powell 1992) and the *Ficus* species and their wasps being exceptions as opposed to the norm. There is general consensus that the terms “generalisation” and “specialisation” create a false dichotomy; and in reality pollination systems of plants can be viewed as a continuum ranging from extreme specialization where a species relies on just one or a few closely related species to extreme generalisation whereby any number of plant visitors can effect pollination. This departure from the traditional Darwinian convention of an evolutionary trend towards specialisation weakens the reliability of “pollination syndromes” as a means to predicting plant-pollinator relationships (Ollerton 1998) and has important implications when attempting to assess risk of ‘mutualism failure’, a risk that has engaged growing concern amongst conservationists in recent years (Bond 1994, Kearns et al. 1998).

In dispersal systems, it is widely agreed that the trend towards generalisation is relatively more pronounced, with no known examples of obligate mutualisms between plant and disperser (Wheelwright and Orians 1982, Herrera 2002). Jordano (1987) calculated the average number of interactions between plants and their pollinators or dispersers for 36 pollination and 19 seed-dispersal studies and found that average number of interactions per plant species was higher by c.50% in dispersal (6.0) versus pollination systems (4.1). This difference can be attributed to the fact that pollen and seeds have very different ‘targets’ and therefore different selective pressures driving co-evolution. The target for pollen is quite specifically the receptive stigma of a conspecific plant and a pollinator will not receive reward unless this target is reached, however the target for a fruit is any suitable microsite for establishment (Howe and Westley 1986). As the disperser has already received reward in advance in the form of a fleshy fruit, intuitively it is very difficult to then direct a disperser to a specific microsite. Mistletoe and other cases of ‘directed dispersal’ are exceptions to this rule, and in these cases the increased advantage of specialisation may provide the selective pressure for more specialised plant-disperser relationships to coevolve (Howe and Smallwood 1982, Reid 1989, 1991, Wenny and Levey 1998, Green et al. 2008).

As the biology of reproductive mutualisms has been described as “notoriously complex” (Bond 1994, p 83), it is important to establish a thorough understanding of the natural history of

individual species and their pollination and dispersal systems when attempting to understand the importance of these interactions in species persistence. Only once we have a clearer picture of these interactions can we begin to build a picture of entire plant-pollinator and plant-disperser networks within and across ecosystems and hope to disentangle the complexity of the “entangled bank”.

1.2 Mutualisms in Afromontane forests

The Afromontane region, often referred to as the ‘Afromontane archipelago’ can be recognised as seven disjunct mountain systems isolated within a matrix of surrounding lowland (White 1978) (Fig 1.1). Within the Afromontane region, forests comprise the lowermost vegetation type occurring between 990 – 4000 m a.s.l depending on latitude and proximity to sea. Despite their disjunct distribution these Afromontane forests are characterized by high endemism and a cohesive floral composition that is markedly distinct from that of surrounding lowland forests (White 1981, White 1983). Afromontane forests currently face the same threats as those confronting tropical forests worldwide (Chapman et al. 2004), however many species and their ecological interactions such as pollination and dispersal remain poorly understood due to the paucity of published studies (Grimshaw 2001).

1.2.1 Afromontane pollination mutualisms

Rodger et al (2004) reviewed pollination biology studies across Africa and concluded that very little research has been conducted outside of the Cape region. Overall, the majority of studies that have been carried out are of an evolutionary nature with just 23% involving experimental manipulations and only 3% addressing conservation application. Furthermore, indices of ‘percentage of intensity of study’ indicate that of the 20 largest families on the continent, only three have been studied in any depth - Iridaceae, Proteaceae and Geraniaceae (Rodger et al. 2004). It is therefore not surprisingly that published pollination work in Afromontane forests is restricted to two observational studies by Kirkup (1984) and Dowsett-Lemaire (1989). Both of these studies indicate that nectivorous birds play an important role in the pollination of Afromontane forest species.

Kirkup (1984) made observations of avian visitors to two Loranthaceous mistletoe species (*Tapinanthus bangwensis* and *Globimetula braunii*) in open bushland along the edges of

Afromontane forest in the Bamenda Highlands, Cameroon. Three species of sunbird (the yellow-bellied sunbird (*Nectarinia venusta*), Preuss's double collared sunbird (*N. preussii*) and the splendid sunbird (*N. coccinigaster*) were observed feeding at the mistletoe on both mature buds and previously opened flowers, with the latter being the more commonly observed. Weaver birds (Ploceidae) were observed robbing nectar from closed buds of *T. bangwensis*. Kirkup (1984) proposed that in certain areas, *G. braunii* may gain some assurance of flower visitation by becoming a temporally exclusive food source for the sunbirds, after which time the birds migrate to other areas.

Dowsett-Lemaire (1989) investigated nectar-foraging and the annual cycles within a montane community of six sunbird species on the Nyika Plateau, Malawi. The role of the sunbirds versus insects in pollinating 40 plant species was investigated. An almost equal number of species were either insect (n = 16) or sunbird (n = 17) pollinated, whilst the remaining seven plants appeared to be pollinated by both insects and sunbirds. Flowers fitted two distinct morphological syndromes. Insect-pollinated plants had white, blue, purple or yellow flowers with an open cup or short tubes (2-15 mm) whilst sunbird-pollinated flowers were usually orange-red and odourless, with long narrow corolla tubes 12-35 mm in length. Flowers pollinated by both insects and birds were morphologically variable. All six mistletoe species observed were exclusively sunbird-pollinated.

Currently work is underway in Ngel Nyaki Forest Reserve, Mambilla Plateau to construct a 'pollination web' (Memmott 1999) of all tree species in the reserve (K. Poloma, pers. comm.). This work will boost significantly the current knowledge of pollination mutualisms in Afromontane forests.

1.2.2 Afromontane dispersal mutualisms

As for most tropical flora (Howe and Smallwood 1982), the majority of Afromontane forest species rely on animals for seed dispersal (85% Nyika Plateau, Malawi; 81.6% Kilum-Ijim forest, Cameroon; 80% Mafai, Tanzania) (Dowsett-Lemaire 1985, Maisels and Forboseh 1999, Lyaruu et al. 2000 respectively). Studies in the Afromontane forests of Malawi, Nigeria and Cameroon attribute most animal-mediated seed dispersal to frugivorous birds and to a lesser extent, monkeys (Dowsett-Lemaire 1988, Maisels and Forboseh 1999, Ihuma 2006).

Birds were found to comprise 94% of the frugivorous fauna in the Nyika Plateau forests of Malawi (Dowsett-Lemaire 1988) and 76 % in Ngel Nyaki forest, Nigeria (Ihuma 2006). Furthermore, in the Kilum Ijim forest of Cameroon, Maisels and Forboseh (1999) recorded 82% of woody species as being primarily adapted to seed dissemination by birds. The collection of fruits in these forests comprise predominantly of fleshy berries and drupes, that are mainly red, purple-black, brown or green in colour and relatively small sized between 3-19 mm in diameter. In Malawi, fruits larger than 19 mm in diameter account for 25% of the recorded 114 species with berries and drupes (Dowsett-Lemaire 1988).

Dowsett-Lemaire (1988) indicated high dietary overlap between most Afromontane bird species and found that 90% of woody bird-dispersed plants are also dispersed by the blue monkey *Ceropithecus albogularis*. High selectivity in the feeding of the pigeon *Columba arquatrix* was recorded however, which showed a strong preference for oily drupes and fibrous capsules and is thought to play an important role in long-distance seed dispersal. Dowsett-Lemaire (1988) noted one example of a highly specialised relationship; that between the tinkerbird *Pogoniulus leucomystax* and the mistletoes upon which it feeds. She found that the tinkerbird fed on the berries of parasitic mistletoes (Loranthaceae/ Viscaceae) all year round eating few other fruits species and, reciprocally, the mistletoe berries are largely ignored by other bird species.

Fruit size rather than quality appears to be the main determinant of the number of frugivores exploiting a fruit crop. Small-fruited species attract significantly more frugivores than large-fruited ones due to the constraints that gape-size imposes on consumption of larger fruits by smaller birds, and the fact that larger birds commonly feed on small fruits. Larger fruits (>13-15 mm in diameter) are restricted to dispersal by larger-gaped dispersers, such as hornbills, pigeons, turacos and primates (Dowsett-Lemaire 1988).

It is predicted that as disparity in fruit size increases, so too will the dissimilarity in frugivore assemblage between different tree species (Wheelwright 1985b). This prediction was supported in a study by Githiru et al. (2002) on the effects of fruit size and site on the composition of avian frugivore assemblages in a fragmented Kenyan cloud forest. Githiru et al. (2002) found that although the frugivores in this forest system showed little specialisation, frugivore assemblages were generally more similar on tree species with fruits of a similar size at the same site. Frugivore assemblages were also found to be more similar on conspecific trees occurring at the same site and within, rather than between fragments, suggesting limited frugivore movement

between different forest patches. These findings indicate that whilst specialised dispersal mutualisms may be rare, changing land use resulting in habitat disturbance and fragmentation may affect fruit selection thereby changing patterns of seed dispersal and regeneration in montane forests (Githiru et al. 2002).

Despite high overlap in diet, different dispersers can vary considerably in the quality and quantity of dispersal service provided, resulting in considerable variation in the effectiveness of different animals as seed dispersers (Schupp 1993). Two studies in the Nyungwe Montane Forest, Rwanda examine the variation in dispersal ability of closely related bird species.

Graham et al. (1995) examined the effectiveness of two common bulbuls (*Andropadus latirostris* and *A. tephrolaemus*) in dispersing the seeds of an African montane pioneer forest tree *Maesa lanceolata*. Quantity of dispersal was measured in terms of disperser abundance, tree visitation frequency, number of fruits taken during a visit and number of seed defecations away from the parent tree. Quality of dispersal was assessed by examining seed viability after passing through the digestive system of the bulbuls, deposition rate and diversity of daily feeding pattern. Results showed that both bulbul species were effective in dispersing 90 – 95 % of seeds away from the parent plant. However, *A. tephrolaemus* was proposed to be a more important disperser of *M. lanceolata* seeds due to its higher visitation rate (Graham et al. 1995).

Sun et al. (1997) subsequently investigated the relative effectiveness of seed dispersal by three turaco species (The great blue turaco (*Corythaeola cristata*), the ruwenzori turaco (*Musophaga johnstoni*) and the black-billed turaco (*Turaco schuettii*)). Two elements affecting the quality of seed dispersal were investigated; the probability that an ingested seed will be dispersed away from the parent tree and the distances seeds are dispersed. By observing focal birds in the wild and measuring captive gut retention times, seed shadows could be calculated for each turaco species. Due to its long gut retention time and typically longer flight distances, the great blue turaco dispersed seeds the greatest distance on average and over a wider variety of habitats. The ruwenzori turaco deposited the highest percentage of ingested seeds away from the parent tree as it had the shortest residence time in feeding trees; however it also dispersed seeds the shortest distance partly because of its short flight distances. The black-billed turaco fed least frequently and had the longest time interval between successive feeding bouts; consequently, it deposited seeds more evenly among perching sites relative to the other two turacos. Despite these variations

in the way the three turaco species dispersed seeds, each was able to disperse more than 80% of ingested seeds away from the parent tree (Sun et al. 1997).

The majority of studies assessing the role of primates as seed dispersers in Afromontane forests focus on the *Cercopithecus* African forest monkeys, commonly named guenons (Beeson et al. 1996, Kaplin and Moermond 1998, Kaplin et al. 1998, Kaplin and Moermond 2000). In the Afromontane forests of Cameroon and Rwanda, fruit and seed items comprise approximately half of the guenon diet throughout the year (Beeson et al. 1996, Kaplin et al. 1998). Kaplin and Moermond (1998, 2000) compared variations in seed handling of the blue monkey *C. mitis doggetti* with the more terrestrial L'Hoest's mountain monkey *C. l'hoesti* in Nyungwe Forest Reserve, Rwanda. These studies were the first to examine the role of the L'Hoest's mountain monkey in seed dispersal. Examinations of dung samples revealed 100% of *C. l'hoesti* and 94% of *C. mitis doggetti* dung samples contained intact seeds, and that the seeds were greater in number and larger-sized than reported elsewhere for African *Cercopithecus* monkeys (Wrangham et al. 1994). *C. mitis doggetti* was observed to consume twice as much fruit as *C. l'hoesti* (comprising 50% and 25% of the monkeys' diet, respectively) yet on average, *C. l'hoesti* dispersed significantly more seeds, particularly larger seeds >2 mm in diameter.

Chimpanzees *Pan troglodytes* are often the largest primates in African forests and are known to be effective seed dispersers (Wrangham et al. 1994, Lambert 1999). Estimates of seed dispersal for some chimpanzee populations amount to 369 large (>2 mm) seeds km⁻² day⁻¹, or per individual, 147 large seeds km⁻² day⁻¹ (Wrangham et al. 1994).

Basabose (2002) observed chimpanzees inhabiting the montane forest of Kahuzi in the Democratic Republic of Congo over seven years and found they consumed 66 different fruit species. However fecal sampling over the same period showed their diet to be very selective, with the seeds of only a few pulp-fruit species being regularly recorded. Basabose (2002) proposes that the low diversity of fruits consumed regularly by the Kahuzi chimpanzees reflects the limited range of fruits available within the Kahuzi Montane forest relative to tropical rainforests at lower altitude. Fecal analysis revealed that fig fruits were the most frequently eaten fruit species, the seeds of which occurred in 92% of a total of 7,212 fecal samples (Basabose 2002).

A recent study by Gross-camp and Kaplin (2005) examined the postdispersal fate of large seeds (≥5 mm) following dispersal by chimpanzees in an Afromontane forest in Rwanda.

They assessed the persistence and germination of seeds in chimpanzee faeces and wadges (orally discarded fruit mass) against six different microhabitat characteristics.

A higher proportion of seeds persisted at deposition sites with higher elevation, a result which may be attributed to fewer seed predators or secondary dispersers at higher altitudes (Gross-Camp and Kaplin 2005).

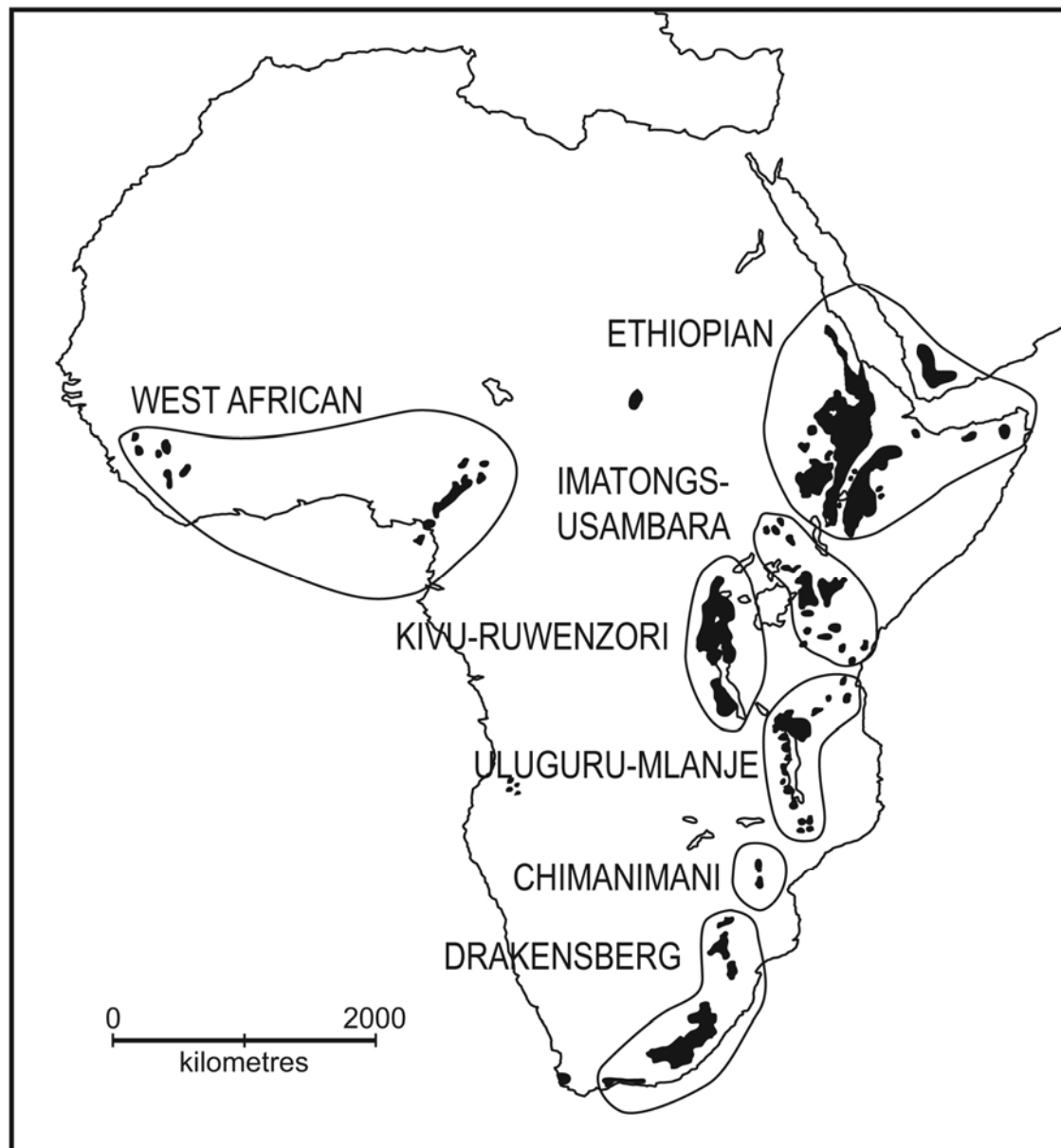


Fig 1.1: The seven regional mountain systems comprising the Afromontane archipelago (shown as solid black areas). Adapted from White (1978).

1.3 Introduction to the Mistletoes

Mistletoes are a diverse group of widely distributed hemiparasitic flowering plants comprising over 1300 species within the order Santales (Calder 1983, Watson 2001). They are termed hemiparasites because although most mistletoe species photosynthesize, they obtain all water and minerals from their host via a specialized vascular attachment called a haustorium (Calder 1983). Taxonomic treatment of the group has long been disputed (Engler 1889, Van Tieghem 1895, Danser 1933, Kuijt 1968, Kuijt 1969, Weins and Barlow 1971, Calder 1983), however recent phylogenetic work confirms the mistletoes belong to five distinct families; Misodendronaceae, Eremolepidaceae, Santalaceae, Viscaceae and Loranthaceae (Der and Nickrent 2008, Malecot and Nickrent 2008, Vidal-Russell and Nickrent 2008b).

Together, the Misodendronaceae, Eremolepidaceae and Santalaceae comprise the minority of all mistletoe species (<2%), with the two former being restricted to neotropical forests whilst Santalaceae is also present in Latin America and Southeast Asia (Kuijt 1969, Watson 2001). In contrast, Viscaceae and Loranthaceae include the vast majority of species and are both widely distributed across all continents except Antarctica, and have many endemic representatives on oceanic islands (Barlow 1983).

Species from 97 vertebrate families have been recorded as consuming mistletoe, the majority of which (over 80%) were recorded feeding on nectar and/or fruits. Due to the high incidence of these and other important mistletoe-animal interactions, mistletoes are considered to function as a keystone resource in many forests and woodlands worldwide and serve as an effective model for the study of plant-animal mutualisms (Watson 2001).

1.3.1 Loranthaceae

The Loranthaceae comprise the largest group of mistletoes, with over 900 species in 73 genera distributed widely from the tropics to temperate regions, particularly in the southern hemisphere (Vidal-Russell and Nickrent 2008a). Most members of the Loranthaceae are stem-parasites, but in three genera, primitive root parasites also occur – *Nuytsia*, *Atkinsonia* and *Gaiadendron* found in Western Australia, Southern Australia and South America respectively (Kuijt 1969). Although many species are able to spread vegetatively within their host tree by the growth of epicortical roots or elaborate systems of spreading bark strands, spread between host trees is not possible and the mistletoe will generally die with the death of its host (Kuijt 1969).

Outbreeding is considered the norm within the family (Barlow 1983). Self-compatibility has been identified within several genera (Docters Van Leeuwen 1954, Bernhardt 1983, Rivera et al. 1996, Vaknin et al. 1996, Ladley et al. 1997, Aizen 2005, Azpeitia and Lara 2006).

The flowers of Loranthaceous mistletoes are generally hermaphrodite (Barlow 1983) however unisexuality does occur within a few genera and is always associated with either dioecy (having separate male and female plants) or subdioecy (Kujit 1969, Ladley et al. 1997).

The corolla is 4-6 merous, occasionally nine and usually actinomorphic (radially symmetric) (Kujit 1969). Floral syndromes within the group are consistent with insect or bird pollination; the former characterized by small (2-10 mm) white or greenish flowers whilst the more common bird-pollinated syndrome is distinguished by typically long (30-120 mm), odourless and brightly coloured (red, yellow, orange) flowers which contain copious sugar-rich nectar (Kujit 1969, Vidal-Russell and Nickrent 2008a).

The ovary is inferior and does not have true ovules, and thus technically, also lacks true seeds. However, the term seed is applied to the functional unit. The fruits of the Loranthaceae are generally small and berry-like with a single embryo (occasionally two) surrounded by a viscin layer, a sticky substance derived from the fruit wall which serves to anchor the seed to a host branch when voided (Kujit 1969).

Reproductive mutualisms within the family range in specificity, with more specialised relationships typically recorded between the Loranthaceae and their dispersers (Godschalk 1983b, Reid 1989, 1991, Martinez del Rio et al. 1996, Amico and Aizen 2000). (See sections 2.2 and 3.3 for reviews of Loranthaceae pollination and dispersal mutualisms, respectively).

1.3.2 African Loranthaceae

Of the 73 recognised genera of Loranthaceae worldwide (Vidal-Russell and Nickrent 2008a), 21 (29%) occur in Africa, with the majority (90%) of these being endemic to Africa, or Africa and the Arabian Peninsula (Polhill and Wiens 1998).

More recent taxonomic work on African Loranthaceae has focused on cytology and biogeography of the family, resulting in the currently recognised division of the family into 21 distinct genera comprising over 200 species (Barlow and Wiens 1971, Polhill 1989, Polhill and Wiens 1998).

All known African Loranths are small, short-lived aerial parasites growing on their woody hosts, with no terrestrial genera recorded. The haustoria display a wide variety of forms between the different genera, ranging from species with one primary haustorium to those with epicortical roots or elaborate systems of spreading bark strands (Calvin and Wilson 1998). Host specificity is generally low, with 73% of African Loranthaceae occurring on many different plant species and genera in a wide range of families (Polhill and Wiens 1998).

With the exception of a few species from the genus *Helixanthera*, all African Loranthaceae have hermaphroditic flowers primarily adapted for pollination by Sunbirds (Nectariniidae) – the principal nectar-feeding birds of Africa (Polhill and Wiens 1998). Whereas flower structure in Loranthaceae elsewhere is generally actinomorphic (radially symmetric), in African members it is predominantly zygomorphic (bilaterally symmetric) (Kujit 1969). Floral diversity amongst African Loranths is immense and specialisation, marked by differences in signals given to visiting birds, is reported to have reached the highest levels in the family here (Barlow 1983, Polhill 1989, Kirkup 1993). In contrast, the fruits and seeds are relatively uniform and are consumed predominantly by the tinkerbirds (*Pogoniulus* spp.), the main agents of mistletoe dispersal in Africa (Dowsett-Lemaire 1982, 1988, Godschalk 1983). (See sections 2.3 and 3.3 for reviews of African Loranthaceae pollination mechanisms and dispersal mutualisms, respectively).

The African people have numerous myths and legends around the mistletoes which on the Mambilla plateau, are known as ‘Sutore’ (pronounced soh-tor-ray). For example, it is believed that mistletoes found naturally growing on certain plants will bring great wealth and prosperity. This belief may be a reflection of the specialised relationship often observed between mistletoes and their host plants and the rarity of finding mistletoe growing on certain plant species. The fruits of mistletoes are also used as an effective trap by local African villagers on the Mambilla plateau to capture birds. The viscid ripe fruits are collected from the plants and boiled with water until the mixture becomes black and very sticky. This mixture is then smeared onto the branches and ironically the very birds whom might otherwise disperse the fruits become stuck by their legs or wings. This technique is practiced by many tribes in the area such as the Mambilla, Kaka, Ndoro and Panso tribes (M. Zuibaru, pers. comm.).

1.4 Mistletoe species studied

The three Loranthaceae species studied in this thesis are endemic to tropical Africa and have been the subject of little previous research. They are all members of the Tapinanthoid group within the genera *Globimetula* and *Agelanthus*, the latter being the largest genus of the African Loranthaceae. The reproductive ecology remains largely unstudied for all three species across their natural range.

1.4.1 *Globimetula braunii* (Engl.) Tieghem

Stems of *G. braunii* are usually 0.5 – 2 m, but can be up to 4 m, and are generally from a single attachment, though numerous secondary haustoria have been observed. Leaves may be opposite, subopposite or sometimes ternate (in Zaire basin) and are elliptic with a blunt or rounded apex (4 -10 cm x 1.5 - 7 cm). Flowers occur in umbels of 3 - 6, are usually 2 – 4.5 cm long, red or pink in colour and have an apical swelling which darkens upon ripening. Sunbirds are the only flower visitors previously recorded for this species and are thought to be the only legitimate pollinators (Kirkup 1984, 1993). The berry is orange or red (5 – 6 mm x 5 mm) and contains an orange or red seed (3 x 1.5 mm). The fruits of *G. braunii* are thought to be dispersed by tinkerbirds (R. Polhill, pers. comm.).

G. braunii is the most widespread member of the genus, ranging in the Guineo-Congolian forests from Ivory Coast to Southern Sudan and Western Kenya, and also throughout the Congo basin. Host species are varied and include plantations of citrus, cocoa, coffee and rubber. There are thought to be many local races and much variation within (Polhill and Wiens 1998).

In Nigerian folklore medicine, *G. braunii* leaves are used in the treatment of hypertension, rheumatism, epilepsy, infertility, stomach problems, digestive aid, diabetes, pregnant labour and as a laxative (M. Zuibaru, pers. comm.). Recent studies undertaken on laboratory rats confirm the medicinal qualities of *G. braunii* (Olagunju et al. 1999, Fred-Jaiyesimi et al. 2008, Ie and Zam 2008). Olagunju et al. (1999) studied the anti-diabetic properties of *G. braunii* in alloxan-induced diabetic rats and found that leaf extract taken from plants parasitizing cocoa trees (*Theobroma cacao*) contained hypoglycaemic, hypocholesterolaemic and hypolipidaemic principles. Fred-Jaiyesimi et al. (2008) recently reported a laxative effect on laboratory rats fed leaf extract from *G. braunii* plants parasitizing *Cola acuminata*. Ie and Zam (2008) confirmed

the oxytocic (labour inducing) effects of *G. braunii* leaf extract from plants parasitic on *Citrus sinensis*.

1.4.2 *Agelanthus brunneus* (Engl.) Danser

Stems of *A. brunneus* grow up to approximately 1 m with alternate to opposite and ternate leaves, elliptic to oblanceolate in shape, rounded at the apex and largely variable size 2.5 – 13 cm x 1.5 – 8 cm. Flowers are few to numerous, crowded at axils and at older nodes and not in obvious umbels. Corollas are 3.2 – 4.5 cm long with various colourings of white to pink or green to yellow and orange, dark-tipped and all with several colour bands near apex and over vents. There is a characteristic and obvious basal swelling of the corolla tube from young bud-stage which helps distinguish *A. brunneus* from *A. djurensis* and other closely related species. The pollinating agents of *A. brunneus* are unknown, but are presumed to be sunbirds (R. Polhill, pers. comm.). The smooth berry is obovoid to urceolate, 7 – 8 x 4.5 – 5 mm in size. Although the fruits of African Loranths are primarily dispersed by tinkerbirds (Dowsett-Lemaire 1982, 1988, Godschalk 1983), Eastern chimpanzees (*Pan troglodytes schweinfurthii*) have been observed feeding on *A. brunneus* fruits in the montane forest of Kahuzi-Biega National Park, Democratic Republic of Congo (Basabose 2002).

A. brunneus is widespread in Guineo-Congolian forests from Senegal to Uganda and western Kenya and south to northern Angola (Polhill and Wiens 1998). It is known to grow on a wide variety of hosts, including rubber trees, and can be found flowering at different locations during most months of the year (Begho et al. 2001).

1.4.3 *Agelanthus djurensis* (Engl.) Danser

A. djurensis is a glabrous shrub with stems 0.5 – 2 m long. Umbels of 3 – 6 flowers occur at axils and older nodes. The corolla is 4 – 5 cm long, deep crimson or wine-red (sometimes paler yellow-green when young), paler over vents and dark-tipped. There is no or very little basal swelling of the corolla. The berry is red or white, obovoid and 9 x 6 mm in size. Pollinating and dispersal agents for this species have not yet been recorded.

A. djurensis is distributed in forest regions from Cameroon to Sudan and Uganda, and extends south to Angola in areas with more seasonal rainfall than those occupied by *A. brunneus* (Polhill

and Wiens 1998). This species has not previously been recorded in Nigeria (R. Polhill, pers. comm.).

1.5 The Study Site – Ngel Nyaki Forest Reserve

Ngel Nyaki Forest Reserve is located between 1400 – 1600 m elevation on the Western Escarpment of the Mambilla Plateau, South East Nigeria (07°14' N, 011°04' E) (Fig 1.2). The reserve is approximately 46 km² in area, 7.2 km² of which comprises one of the most floristically diverse submontane - montane forest stands in Nigeria. The diversity of the forest flora is reflected in the richness of the fauna, particularly primates including the Nigerian chimpanzee (*Pan troglodytes vellerosus*), putty nose monkeys (*Cercopithecus nictitans*) and tantalus monkeys (*Chlorocebus tantalus*). The reserve is also an important bird area (Chapman and Chapman 2001, Chapman et al. 2004).

Despite the forest being gazetted as a Local Government Reserve in 1969, hunting pressure and the infringement of local subsistence farmers into the reserve for slash and burn agriculture is causing physical damage to the forest and soil erosion along its boundary (Chapman et al. 2004).

The climate of the Mambilla Plateau is highly seasonal, consisting of a dry season from November to March, followed by a rainy season from April to October. Monthly mean temperatures do not exceed 30°C and annual rainfall is > 1,780 mm, falling almost entirely during the rainy season (Chapman and Chapman 2001).



Fig. 1.2: Map showing location of Ngel Nyaki Forest Reserve (3) on the Mambilla Plateau, South East Nigeria. Chapman & Chapman, 2001

1.6 General objectives of this thesis

The overall objective of this study is to investigate the importance of plant-animal mutualisms to the reproductive ecology of three West African mistletoe species, *Globimetula braunii*, *Agelanthus brunneus* and *Agelanthus djurensis*, in Ngel Nyaki Forest Reserve, Nigeria.

In order to achieve this objective, for each mistletoe species I aim to:

1. Identify their biotic pollinators and investigate the relative role of different pollinator taxa in plant reproduction
2. Develop an understanding of the floral ecology, including pollination and breeding systems
3. Investigate the dispersal ecology (where possible)

Chapter 2: Floral Ecology: Pollination and Breeding Systems

2.1 Introduction

The importance of animal-mediated pollination to a plants' reproductive ecology is inextricably linked to its breeding system. There are three basic breeding systems in plants: cross-pollination, self-pollination or asexuality. Breeding systems are rarely fixed or static and can respond to changing selection pressures. Many plant populations have a mixed reproductive strategy where reproduction can occur through selection for at least two, and often all three of these strategies, thereby reducing reproductive dependence on mutualisms (Bond 1994). For example it is thought that the shift from animal to wind or self-pollination has evolved in many angiosperm taxa at times when animal pollinators are limited (Cox 1991, Schoen et al. 1996, Culley et al. 2002). This labile nature of breeding systems can lead to regional patterns such as those observed at higher latitudes and altitude, where climatic uncertainty may be greater and environmental stresses promote abiotically or self-facilitated reproduction (Regal 1982, Sobrevila and Arroyo 1982, Bawa 1990, Peck et al. 1998, Medan et al. 2002). The transition from outcrossing to predominantly selfing has occurred in a wide variety of plant groups and is one of the most frequently observed evolutionary pathways in flowering plants (Stebbins 1950, Goodwillie et al. 2005). However this transition is accompanied by the risk that if a breeding system is lost from a population, it may not be recaptured. This has serious implications if selective pressures change under future environmental conditions. The prevalence of cross-pollination throughout plant populations indicates that the fitness advantages of sexuality (i.e. maintained or increased genetic variability) outweigh the benefits bestowed by selfing and asexual reproduction to the extent that outcrossing is maintained as a reproductive option for the vast majority (Charlesworth and Charlesworth 1987, Goodwillie et al. 2005). To this end, many plants have evolved genetically based self-incompatibility mechanisms to prevent self-fertilisation, whilst others exhibit temporal (dichogamy) or spatial separation (herkogamy) of pollen presentation and receipt, or a dioecious mating system where male and female flowers are on separate plants to prevent selfing (Richards 1996).

Reduced reproductive output (in terms of fruits and/or seeds) may be attributed to an inadequate quantity or quality of pollen being delivered to flowers, a phenomenon commonly termed 'pollen limitation'. This may result from a reduced pool of pollinators visiting flowers, smaller pollen

loads per visit, or when poor-quality incompatible pollen (from the same plant or a different species) is delivered (Ashman et al. 2004). A review of 482 studies assessing fruit production in pollen supplementation experiments reported 62% of species exhibited pollen limitation during some years or at some locations (Knight et al. 2005). One way that obligately outbreeding plants can reduce the risk of pollen limitation is by maintaining a generalised pollination strategy attracting a wide array of generalist pollinators. Alternatively, selective pressures resulting from pollen limitation within a population may give rise to the evolution of adaptive traits targeting specific types of pollinators. For example, plants exhibiting a pollination ‘syndrome’ or suite of floral traits commonly associated with ornitholophily (pollination by birds) are often common at relatively high altitude, where birds serve as more effective pollinators in the cool and wet conditions typifying those regions (Cruden 1972, Stiles 1978, Regal 1982, Sobrevila and Arroyo 1982, Bawa 1990, Devy and Davidar 2006). Specialisation may also provide a selective advantage over generalisation by reducing competition in species-rich habitats and excluding ‘illegitimate’ flower visitors which may otherwise ‘rob’ nectar or pollen without actually effecting pollination (Proctor et al. 1996, Kirkup 1998).

In addition to flower traits such as colour, odour and structure, floral rewards such as nectar play an important role in the evolution of plant-pollinator mutualisms (Grant 1950, Van der Pijl 1961, Raven 1972). Plants can effectively manipulate the behaviour of their pollinators by regulating nectar production to produce just enough nectar per flower to attract pollinators, but not enough to satiate pollinators before they have visited flowers on multiple plants. The adaptive explanation for this is that geitonogamy (the fertilisation by pollen from flowers on the same plant) is effectively reduced and outbreeding maximised (Snow et al. 1996). Timing of nectar production can also affect the behaviour of pollinators. For example if the majority of nectar is produced in the mature unopened bud, then opening closed buds presents a large and assured reward to pollinators (Gill and Wolf 1975, Kirkup 1984, Ladley et al. 1997).

Colour changes of flowers post-pollination or retention of old flowers are also adaptive strategies which plants may employ to manipulate the visitation behaviour of pollinators (Stratton 1989, Kirkup 1998).

2.2 Pollination mutualisms within the Loranthaceae

2.2.1 Worldwide

All Loranthaceae are biotically pollinated, and most have large, brightly coloured (red, yellow, orange) hermaphroditic flowers pollinated primarily by birds (Kujit 1969, Vidal-Russell and Nickrent 2008a). Insect-pollinated species occur within a few genera, such as *Tupeia* (Smart 1952), *Ileostylus* (Menzies 1947) and *Barathranthus* (Docters Van Leeuwen 1954). These are usually dioecious or subdioecious with small (2-10 mm) white or greenish flowers (Kujit 1969, Ladley et al. 1997). Pteropodid bats have been recorded visiting the flowers of some Loranthaceae (Fleming and Muchhala 2008), however the role of bats in mistletoe pollination is largely unknown. There are no confirmed records of any other mammal species pollinating mistletoes (Watson 2001).

Birds are thought to have contributed a significant selective pressure in the diversification of the mistletoes due to the close mutualistic relationships frequently described between the plants and their avian pollinators (Feehan 1985, Reid 1990).

Different assemblages of nectar-feeding birds have been recorded as primary pollinators of Lorantheaceous mistletoes across different biogeographic regions worldwide. In the Neotropics, the hummingbirds (Trochilidae) are by far the most important group of birds associated with pollination (Kuijt 1988, Aizen 2005, Azpeitia and Lara 2006). In the Paleotropics and temperate regions of the old world, the honeyeaters of Australasia (Meliphagidae) (Reid 1990, Ladley et al. 1997), the sunbirds (Nectariniidae) of the African region (Kirkup 1984, Feehan 1985, Polhill 1989) and the flowerpeckers or “mistletoe birds” (Dicaeidae) of Indomalaya are all of principal importance to Loranthaceae pollination (Docters Van Leeuwen 1954, Davidar 1983a).

Nectar production within the family is highly variable, though New Zealand and Australian mistletoes appear to have both the largest nectar volumes and energy production per flower (Table 1). This may be driven by the relatively large size of Australasian mistletoe pollinators and their associated higher energy demand (Paton and Ford 1977, Ladley et al. 1997).

No biotic pollinator is known to depend entirely on mistletoe nectar as a sole food source, although in some areas Lorantheaceous mistletoes provide an exclusive nectar source for their avian pollinators during certain times of the year (Kirkup 1984, Watson 1997, Aizen 2005).

Reciprocally, there is no known instance of a mistletoe species exhibiting dependence on a single biotic pollinator for reproduction across its entire range, although in some populations, decline of avian pollinators presents a threat to reproductive success where plants have been shown to exhibit only limited capacity for self-fertilisation and fruit set in the absence of pollinators (Reid 1990, Ladley et al. 1997, Robertson et al. 1999, Aizen 2005).

2.2.2 Africa

Aside from some members of the primitive genus *Helixanthera*, the African Loranthaceae all have flowers adapted for pollination by birds (Polhill 1989, Polhill and Wiens 1998). Sunbirds are the principal pollinators, however smaller passerines such as white-eyes (Zosteropidae) may also play a secondary role in pollination of some species (Polhill 1989).

Variation in the ability to open flowers has been observed between different sunbird species, with a positive relationship between body size and proportion of flowers opened (Gill and Wolf 1975, Kirkup 1993). This trend can be explained by the generally larger (Table 2.1) and more assured nectar reward offered by closed buds and the greater energetic requirements of larger sunbirds. Complete specialisation on the closed buds appears to be offset by the increasing search costs within and between patches (Gill and Wolf 1975). There is considerable overlap in the mistletoe species foraged by the sunbirds (Gill and Wolf 1975, Kirkup 1984, Dowsett-Lemaire 1989, Kirkup 1993).

On several large-flowered Loranthaceous mistletoes, smaller sunbird species have been observed bypassing the stigma and anthers and extracting nectar by piercing the corolla tube in a practice commonly termed ‘nectar robbing’ (Dowsett-Lemaire 1989). Other short-billed passerines such as Weaver birds (Ploceidae) have also been observed robbing nectar from *Tapinanthus* mistletoe flowers along an Afromontane forest edge in Cameroon (Kirkup 1984).

Table 2.1: Nectar volume and sugar concentration in Loranthaceae flowers

	Nectar in closed bud			Nectar in open flower			
Species	Quantity µl	Sugar% (w/w)	Sucrose equivalents (mg/bud)	Quantity µl	Sugar % (w/w)	Sucrose equivalents (mg/flower)	Ref
Worldwide							
<i>Muellerina eucalyptoides</i>						6.34	1
<i>Amyema miquelii</i>				5.1	36.5	1.98 – 2.15	1,2
<i>Amyema miraculosa</i>				1.3	33.8	0.50	2
<i>Lysiana exocarpi</i>						0.93-1.71	1,3
<i>Amyema pendula</i>				3.7	21.4	0.85-1.26	1,4
<i>Amyema quandang</i>				4.7	24.3	1.07-1.08	1,4
<i>Peraxilla colensoi</i>	50.6	14.3 ± 0.7	5.15 - 9.62	69.1 ± 2.6*		5.80*	5
<i>Peraxilla tetrapetala</i>	35.2 -43.5	12.2 ± 1.6	4.01 - 4.58	35.7 ± 2.9*		4.02 – 4.98*	5
<i>Alepis flavida</i>				2.4 ± 1.6*			5
<i>Gaiadendron poasense</i>					16.0		6
<i>Gaiadendron punctatum</i>					5	0.60	7
<i>Psittacanthus calyculatus</i>				2.34 ± 0.2	22	0.56	8
<i>Tristerix corymbosus</i>					29.7- 30.8		9
Africa							
<i>Phragmanthera</i>							
<i>dshallensis</i>	20	17.5	3.85	3.5	17.5	0.67	10
<i>Tapinanthus bangwensis</i>	4.7	8.3	0.39	0.75**	9.0	0.07	11
<i>Globimetula braunii</i>	3.0	10.4	0.31	0.65**	11.2	0.07	11
<i>Emelianthe panganensis</i>				4**	18	0.8	12
<i>Plicosepalus sagittifolius</i>				12.5**	16	2.1	12
<i>Globimetula oreophila</i>	14.5	12	1.8				12
<i>Oncocalyx rhamnifolius</i>	2.1	17	0.4				12
<i>Englerina</i>							
<i>woodfordioides</i>	9.5	9	0.9	2.6**	14	0.9	12
<i>Oncocalyx sulfureus</i>	1.9	28	0.6	0.8**	31	0.3	12
<i>Agelanthus zizyphifolius</i>	4.6	13	0.6	2.1**	18	0.5	12
<i>Agelanthus sansibarensis</i>	16.7	13	2.1	2.2**	3	0.3	12
<i>Erianthemum occultum</i>	3.3	16	0.5	10.2**	13	1.4	12
<i>Erianthemum dregei</i>	6.8	14	1	2.5**	8	0.3	12
<i>Phragmanthera usuiensis</i>	21.6	9	2.1	2**	4	0.2	12

Where ranges are given the values are means from two different data sets

* total production (± standard error) over lifetime of flower

** flowers were open and accessible to pollinators throughout measurements.

References: 1: Reid (1986), 2: Bernhardt (1984), 3: Ford (1979) 4: Bernhardt & Calder (1981), 5: Ladley et al.

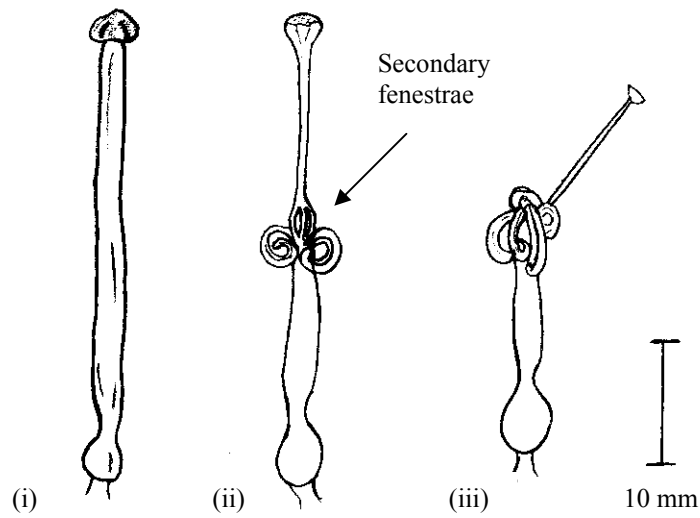
(1997) 6: Wolf et al. (1976), 7: Stiles & Freeman (1993), 8: Azpeitia & Lara (2006), 9: Chalcoff et al (2006), 10: Gill & Wolf (1975), 11: Kirkup (1984), 12: Kirkup (1993)

2.3 Pollination mechanisms in Loranthaceae

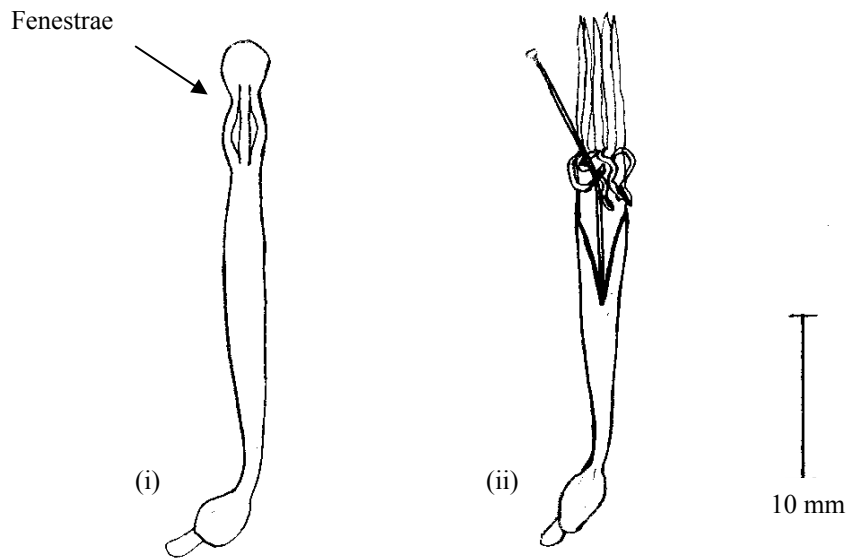
2.3.1 Worldwide

Categorisation of pollination mechanisms in the Loranthaceae fundamentally begins with the distinction between explosive versus non-explosive anthesis. Evans (1895) was the first to describe explosive anthesis in two African mistletoes *Tapinanthus kraussianus* and *Erianthemum dregei*. In this process, pollen is shed from the anthers when they are forcibly released from a buildup of stresses during flower-opening. These stresses are generated by differential growth of tissues as the bud matures (Kirkup 1993). Explosive opening can be obligate, where the flower cannot open unless visited by a biotic agent as in most Loranthaceae (Docters Van Leeuwen 1954, Feehan 1985, Polhill 1989, Kirkup 1993, Ladley et al. 1997), or facultative, where the flowers may be opened by pollinators, but generally open unaided as in, for example, the explosive Indomalayan species *Scurrula atropurpurea* (Docters Van Leeuwen 1954) and the New Zealand mistletoe *Alepis flavida* (Ladley et al. 1997). In contrast, non-explosive flowers do not shed pollen from the anthers during flower-opening but subsequently, when mechanically disturbed by pollinator activity at the open flower.

A further distinction can be made within both the explosive and non-explosive mechanisms between vented and non-vented flowers (Fig 2.1). In non-vented flowers, the pollinator squeezes, taps or twists the mature bud-head causing the corolla-lobes to recoil. In vented flowers, small splits (fenestrae) form between the petals of the mature bud (Kirkup 1993). When the pollinator (usually bird) probes its bill into the fenestrae to obtain nectar, the resultant pressure breaks the bond between the interlocked corolla lobes causing them to separate. If the opening mechanism is one-stage, the reflexing of the corolla lobes allows the stresses in the stamen filaments to also be released, causing them to spring apart and either inflex or coil involutely. In two-stage mechanisms, flower opening and pollination take place in two separate stages. In the first stage, the pollinator gently squeezes, taps or twists the head of the bud which causes an instant separation and recoiling of the corolla-lobes. This first stage exposes a staminal column with secondary fenestrae at its base. The second stage is completed when the pollinator probes into the secondary fenestrae to reach the nectar, which then releases stresses in the stamen filaments causing them to spring apart, recoil and shed pollen explosively (Feehan 1985, Kirkup 1993).



(a) Non-vented Loranthaceae opening mechanism (i) mature bud (ii) first-stage, (iii) second-stage



(b) Vented Loranthaceae opening mechanism (i) mature bud, (ii) open flower

Fig 2.1: Generalised diagram of a typical non-vented (both one and two-stage) and vented Loranthaceae flower opening mechanism.

Different mechanisms of pollination have been described in Loranthaceae species across the biogeographical range of the family. In the Indomalayan region, the majority of explosive flower-opening species have been described from India (Ali 1931, Ali 1932, Davidar 1983b). Of the six explosive species described by Ali (1932) and an additional two by Davidar (1983b), three are obligate and require manipulation by a bird to open. Docters Van Leeuwen (1954) also described two species that exhibit two-stage obligate explosive flower opening on the island of Java (*Dendrophthoe pentandra* and *Macrosolen cochinchinensis*).

Several references have been made to explosive flower-opening in South American mistletoes, however these remain largely unconfirmed (Johow 1900, Kuijt 1969, Kuijt 1988, Tadey and Aizen 2001). Johow (1900) described the buds of *Tristerix corymbosus* as forming narrow fenestrae in the middle of the flower prior to an explosive type of flower-opening. However the stamens do not recoil and the anthers do not dehisce for several hours after the first opening, indicative of a weakly explosive two-stage pollination mechanism (Kuijt 1988). Tadey and Aizen (2001) subsequently detailed this observation describing the process by which the petals of *T. corymbosus* reflex during anthesis. Kuijt (1988) records the buds of *T. peruvianus* as forming fenestrae in the mid portion of the flower bud. These fenestrae are relatively large and bright red suggestive of bird pollination, however the opening process has not been observed for this species. Kuijt (1969) describes an observation by C. H. Dodson where the opening of a large-flowered Ecuadorian mistletoe was achieved by a hummingbird inserting its beak into the side of the bud and flying upwards thereby "unzipping" the flower. Kuijt (1969) suggests this observation was probably a species of *Psittacanthus*, however later proposes it may in fact have been a *Tristerix* species (Kuijt 1988).

Although explosive opening has not been recorded in any Australian mistletoe species, several New Zealand Loranthaceae have been described as having explosive or weakly explosive flower-opening mechanisms (Ladley and Kelly 1995, Kelly et al. 1996, Ladley et al. 1997). Flowers of two species of *Peraxilla* are obligately explosive, whilst *Alepis flavida* is described as having weakly facultatively explosive flowers which are able to open unaided. The mature buds of all three species form fenestrae and the anthers dehisce inside the mature unopened bud prior to opening. Although the flowers of *Peraxilla* are primarily adapted for bird pollination, they are sometimes opened and pollinated by small *Hylaeus* and *Leioproctus*.

bees (Kelly et al. 1996, Robertson et al. 2005). A fourth extinct New Zealand endemic mistletoe *Trilepidea adamsii* is also thought to have had explosively opening flowers as inferred from structural cues (Ladley and Kelly 1995).

2.3.2 Africa

Within the 21 genera of African Lorantheae most display some form of explosive flower opening, aside from a few species in the *Helixanthera*, *Plicosepalus* and *Emelianthe* genera (Kirkup 1998). The pollination mechanism of African Lorantheae is characterised by strong coiling tension in the stamen filaments which results in a highly explosive release of pollen. Kirkup (1998) proposes that this involute coiling of the stamen filaments distinguishes the African Loranthids from other members of the family worldwide in which the petals may separate or recoil, and the stamens spring apart, but the stamen filaments inflex only slightly and do not coil involutely. According to Kirkup (1998) this distinction between coiling and inflexing stamen filaments forms the basis for definition of truly explosive or weakly explosive mechanisms respectively.

Feehan (1983) reviewed explosive flower opening within six genera of Central African Lorantheae identifying both vented and non-vented one and two-stage opening mechanisms. Kirkup (1993) subsequently carried out a comprehensive study on the structural basis of pollination in 74 species of African Lorantheae.

Members of the genera *Vanwykia*, *Taxillus* and *Septulina* all have flowers which are typically weakly explosive as characterised by the weak inflexing of the stamen filaments. The flowers of all three genera are vented, although *Septulina* also contains non-vented species. The corolla-head is generally swollen and it is thought that flower opening is achieved in one stage by manipulation of the swollen bud head. The *Globimetula* and *Tapianthus* genera both have 2-stage non-vented explosively opening flowers, although *Tapianthus* sensu lato also contains vented types. The head of the mature bud is swollen, markedly so in *Globimetula*, and darkens on maturity providing an important visual cue to pollinators. The two genera differ in that the corolla-lobes in the open flower of *Globimetula* coil revolutely whereas the corolla-lobes of *Tapianthus* flowers are mostly reflexing or erect and open unilaterally resulting in a zygomorphic flower. In both genera, the second-stage of opening is carried out by probing into secondary fenestrae at the base of the staminal column. The style is usually bent forward towards

the pollinator by the involutedly coiling filaments. In *Tapinanthus*, the filaments are notably short and tightly coiling.

The remaining genera of African Loranthaceae are described by Kirkup (1993) as having a vented one-stage explosive opening mechanism, with fenestrae forming either near the base, around the middle or towards the apex of the bud, a feature characteristic of the genus. Probing into the fenestrae causes a unilateral separation of the corolla-lobes and prises the filaments apart which then coil either uniformly or distally in one or more complete revolutions. In both *Agelanthus* and *Phragmanthera*, the coiling filaments catch the style and pull it forwards towards the pollinator, as described for *Globimetula* and *Tapinanthus*.

Colour patterns vary considerably within and between the African genera, but are especially developed within *Tapinanthus*, *Agelanthus* and *Phragmanthera*. In these genera, open flowers display a conspicuous throat pattern, which fades as the flower ages. The stigma, style and filaments may also be contrastingly coloured. In *Agelanthus*, the mature bud typically has three regions of distinctive colour banding around the head, vent and proximal regions of the corolla. A fourth much narrower band usually forms a small colour zone at the base of the fenestrae (see Fig 2.7). These highly refined colour signals, together with a specialised opening mechanism are indicative of adaptation to pollination by specialist long-billed sunbirds.

There has been little work carried out on the breeding systems of the explosive African Loranthaceae. Evans (1895) carried out pollinator exclusion experiments on *Tapinanthus kraussianus* in South Africa by enclosing a branch of 80-100 flower buds within a bag and found flowers neither opened nor set fruit. Kirkup (1993) hand-pollinated *Globimetula braunii* flowers in the Bamenda Highlands, Cameroon – two flowers with pollen from the same flower, two with pollen from a different flower on the same plant and two flowers with pollen from different flowers on a different plant. The styles of the hand-selfed flowers exhibited complete necrosis within 18 hours which may provide some evidence for self-incompatibility, though firm evidence for self-incompatibility mechanisms is lacking. Kirkup (1993) also postulates that in the explosive genera, at the moment of anthesis the stigma is probably not receptive as the stigmatic papillae appear crowded close together, only later separating enough for pollen to lodge between the papillae bases. This temporal separation of the male and female phases of the flower (dichogamy) suggests that whilst pollen is deposited onto the pollinator at the initial visit, it is during subsequent visits to the already opened flowers that deposition of pollen onto the receptive

stigma occurs. The dehiscent anthers of the open flower are also usually positioned so that contact with subsequent flower visitors does not occur.

2.4 Sunbirds (Nectariniidae)

The sunbirds (Nectariniidae) are a large guild of small-medium sized passerines with a distinct long, slender and decurved bill used to feed on nectar, insects and spiders. There are approximately 130 species in 16 genera, distributed widely from Africa through to Asia, with two species reaching Northern Australia. The majority of the group is concentrated in Africa, where 88 species in 11 genera are currently recognised (Cheke and Mann 2001). The African genera were formerly placed in two expanded genera (*Anthreptes* and *Nectarinia*) however, later review of the group led to its reclassification into several smaller genera (Irwin 1999).

Sunbirds occupy a broad range of habitats, from plantations and gardens, open scrub, savannah, coastal scrub and montane forest through to secondary and primary rainforest. Despite the adaptation that many sunbirds have made to human modified landscapes, a majority of species are recorded in primary forest (Borrow and Demey 2001, Cheke and Mann 2001).

A wide variety of flowers are visited by the sunbirds, at least 279 genera in 94 families. The Loranthaceae and Fabaceae represent the most heavily visited plant families visited, each with 11 genera of Sunbirds recorded visiting the flowers (Fleming and Muchhala 2008). Some species or groups of species are associated with particular plants such as the Golden-winged Sunbird *Drepanorhynchus reichenowi* with *Crotalaria* sp. and *Leonotis* spp., however the majority are more generalised and there are no recorded cases of any one sunbird species being associated with a single flowering plant species (Cheke and Mann 2001). Although some species exhibit a preference for the lower strata or upper canopy of the forest, most species move freely between the strata depending on the availability of food.

The birds can effectively transfer pollen between plants on their crown feathers (Feehan 1985, Kirkup 1993), bill (Johnson 1996), tongue-tip (Ollerton 1998, Pauw 1998) and even feet (Frost and Frost 1981, Johnson and Brown 2004).

Nectar is generally extracted from flowers through their long tube-like tongues whilst perching on branches, although hovering has been recorded on exotic plant species which lack perches (Geerts and Pauw 2009). Sunbirds have also been recorded as nectar robbers, piercing the bases

of corollas to access the nectar instead of effecting pollination through direct probing of the flowers (Swynnerton 1916, Ihuma 2006).

2.5 Aims

Knowledge of the floral ecology, including pollination and breeding systems, for African mistletoes is limited, and based largely on incomplete observation and structural investigation. The focus of this section of study is to investigate the importance of plant-pollinator mutualisms to the floral ecology of three West African mistletoe species, *Globimetula braunii*, *Agelanthus brunneus* and *Agelanthus djurensis* in an Afromontane forest habitat.

More specially, for each species I aim to:

1. Record flower development including measurements of nectar production over the lifetime of the flowers
2. Identify flower visitors, their behaviour and relative visitation rates at flowers
3. Determine the relative role of flower visitors in flower opening and reproductive output of the plant, investigate breeding systems and in particular, test for self-compatibility, autogamy and pollen limitation.

2.6 Methods and Materials

2.6.1 Study plants

Study plants were selected within Ngel Nyaki Forest Reserve according to two criteria: that they carried sufficient early flower buds and were accessible with a ladder (generally along the forest edge and within 4 m of the ground). Work on Loranthaceae in New Zealand showed that pollination of plants < 4m from the ground did not differ from those higher in the canopy (Robertson et al. 2008). The location of individual plants was recorded using a handheld Global Positioning System (GPS) and mapped onto a digital satellite photograph using Arcview mapping software (Fig 2.2).

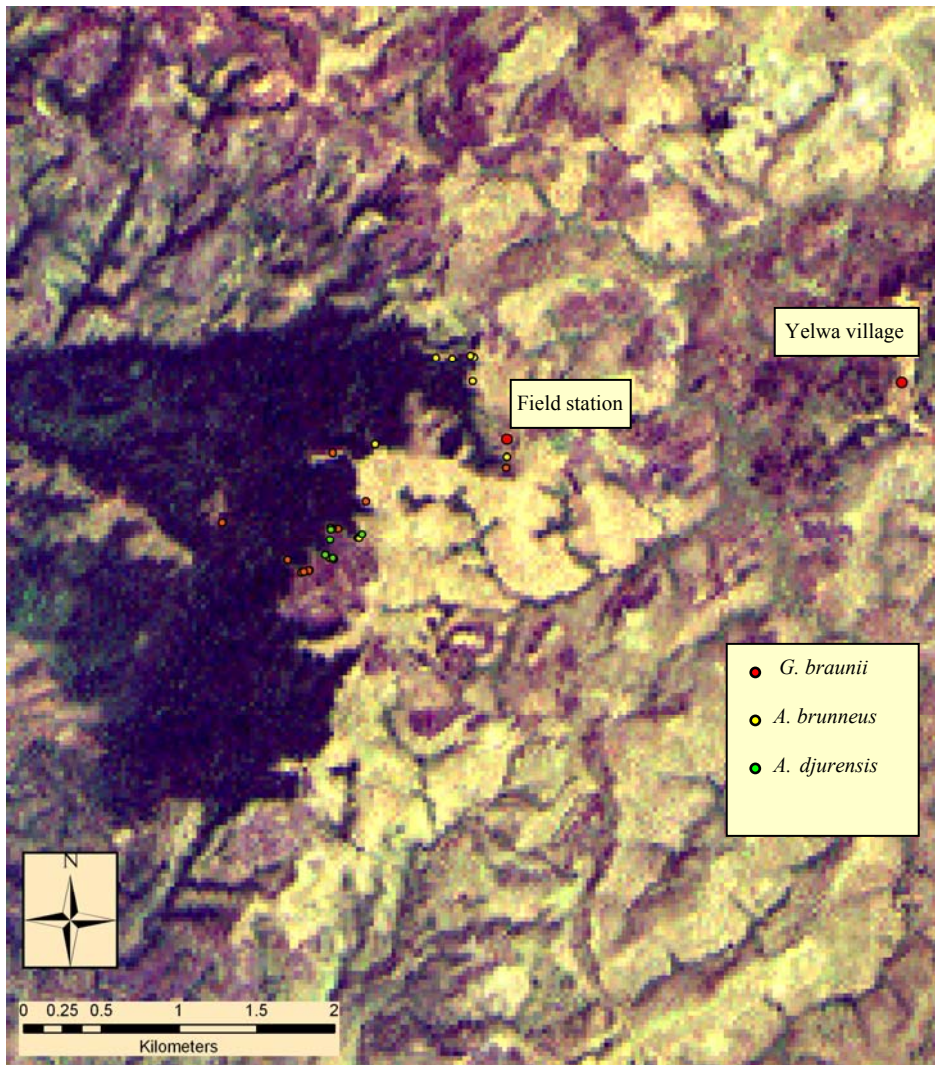


Fig 2.2: Location of study plants within Ngel Nyaki forest reserve, dark area is forest.

2.6.2 Flower development

In the 2007/08 season, *G. braunii* flower development was studied from November 2007 – January 2008 and *A. brunneus* from January – February 2008. In 2008/09, *G. braunii* and *A. djurensis* were studied from November – December 2008. In November 2008, the lifetime of flowers for *G. braunii* and *A. djurensis* was followed by individually tagging flower buds with numbered paper tags and visiting them daily from when the buds first ripened through to when the flowers fell off. Changes in colour and shape of the flowers were also recorded daily. *A. brunneus* was not observed flowering at this time and therefore the lifetime of the flowers had to be estimated from weekly checks for fruit set (see following section 2.6.4 on role of pollinators).

G. braunii

As the bud reaches maturity, the base of the corolla swells and the top half of the corolla flushes a darker shade of pink; this is followed by a darkening of the bud-head from pink to black.

Tapping of the black bud-head causes the corolla-lobes to recoil revealing the staminal column with small fenestrae at the base and anthers still fixed around the red stigma and style-tip. The flower may then remain at this first stage of opening until it abscises and falls from the pedicel, or, probing of the fenestrae causes the corolla-lobes to recoil further thereby releasing tension in the stamens which inroll and expel pollen explosively. The stigma is often, but not always, bent forwards by the incoiling staminal filaments (Figs. 2.3 & 2.4) (See CD enclosed in Appendix 3 for flower opening video footage) (Kirkup 1998, Polhill and Wiens 1998).



Fig 2.3: Scale photo of *G. braunii*. Left – ripe bud, Middle – first stage opening, Right – second stage opening.



Fig 2.4: Development of *G. braunni* bud showing Left – darkening of ripe bud-heads which when tapped causes the petals to recoil, Middle – first stage of opening when petals are recoiled and staminal column with small secondary fenestrae at base are exposed and Right – second stage of opening where stamen filaments have recoiled and pollen has been released explosively.

A. brunneus

The flower buds of *A. brunneus* appear to be polymorphic, with the corollas occurring in different shades of green and pinkish-red at all stages of maturity. Basal swelling of the corolla is present and becomes more pronounced with maturity. There is a distinct colour banding at the top of the bud, with two thin bands of reddish pink separated by a thicker band of white. The upper reddish band blends into the reddish pink tip. When the flower buds are mature, a ‘Chinese lantern’ type structure of fenestrae forms within the white colour band (Fig 2.5). Probing into these fenestrae forms a slit between two of the fused corolla lobes releasing the pollen explosively in one direction. The stigma falls forward in the same direction facilitating contact with the head of pollinators and the anthers release copious amounts of fine white pollen (See Appendix 3 for flower opening video footage). The exposed staminal filaments at the top of the corolla appear bright yellow and form part of a distinctive throat pattern inside the corolla (Fig 2.5). This throat pattern gradually fades with age and the whole open flower lightly flushes pink before abscising (Kirkup 1998, Polhill and Wiens 1998).



Fig 2.5: Left - *A. brunneus* ripe flower buds with strong colour banding around the fenestrae and basal swelling. Above - open *A. brunneus* flowers showing stigma falling forward and distinctive inner-corolla throat colour pattern.

A. djurensis

Structurally, flower buds of *A. djurensis* are similar to *A. brunneus*, though there is no basal swelling and the colouration is very characteristic. *A. djurensis* flower buds are white with dark purple longitudinal stripes, one along each fused lobe (Fig 2.6). The tip of the bud usually becomes dark purple on ripening. Strong colour banding is also present below the tip of the bud, with two thin green bands separated by a thicker band of orange. When the flower buds are mature, fenestrae form within the orange colour band (Fig. 2.7).



Fig 2.6: *A. djurensis*, showing older flowers flushing pink on far right



Fig 2.7: Left – unripe bud, Middle – ripe bud with fenestrae forming, Right – open flower

The opening mechanism is the same as that of *A. brunneus* with probing into the fenestrae causing the flower to open unilaterally in one explosive step (See Appendix 3 for flower opening video footage). The stigma is generally bent and held forward in the same direction by the incoiling staminal filaments rather than just falling forward on opening. The short staminal filaments comprise part of a striking inner corolla throat pattern and the anthers release copious amounts of fine white pollen (Fig 2.8) (Kirkup 1998, Polhill and Wiens 1998).



Fig 2.8: Slightly open *A. djurensis* flower revealing copious amounts of fine white pollen

Nectar production was measured for all three mistletoe species. Unripe buds on four individual plants of *G. braunii* and *A. brunneus*, and two individual plants of *A. djurensis* were enclosed in a nylon mesh bag (50 cm x 40 cm) for the duration of the nectar study to prevent pollinators from opening the buds and harvesting nectar. Buds were checked daily an hour after sunrise, and when sufficient buds for study became ripe on the same day, they were each hand-opened and nectar production per flower was measured using 10 μ l microcapillary. Sugar content of the nectar was also measured in degrees Brix ($^{\circ}$ Bx) (% w/w: weight of sugar per weight of solution) using an Eclipse handheld refractometer. To calculate total mg sugar per flower, percent values of sugar were converted to mg of sugar per μ l using a modified conversion table for sugar concentrations (Galetto and Bernardello 2005). Each bud was covered between measurements and continued to be measured daily an hour after sunrise until either complete senescence of the flower or no nectar had been recorded for 3 consecutive days.

To test for the effect of day after opening on nectar concentration (% w/w), linear regression was performed using the R statistical package (v.2.6.2; The R development Core Team, 2008). Concentration data for *A. brunneus* were log transformed to improve normality.

2.6.3 Pollinator visitation observations

Pollinator observations were carried out on a total of ten *G. braunii* plants from 20 November 2007 – 2 December 2007, eight *A. brunneus* plants from 3 January 2008 to 20 January 2008 and four *A. djurensis* plants from 23 - 24 November 2008, during the peak flowering period of all observation plants. All observations were carried out during the dry season when weather conditions were consistent throughout, with clear skies and a light Harmattan (the West African trade wind). Daily maximum temperatures ranged between 24 – 27 °C, and daily minimum temperatures between 14 – 18 °C. During the study period, the dry season lasted from 18 November 2007 through until 24 February 2008, and from 30 October 2008 to 2 April 2009.

As flowering of both *G. braunii* and *A. brunneus* was asynchronous among plants, finding enough accessible study plants at a comparable stage of early flower to observe on the same day was not possible. Consequently, only two *G. braunii* plants were able to be observed per day and just one per day for *A. brunneus*. Flowering in *A. djurensis* appeared to be more synchronous and consequently four plants were selected to be observed on the same day. Prior to the start of observation (around sunrise at 6 am), the number of all ripe buds and open flowers within a designated field of view, referred to here as the ‘focal area’, was recorded. Size of the focal area varied between plants and was delimited with the aim of achieving a clear field of view to several branches bearing many ripe buds and freshly opened flowers.

All mistletoes were observed through Nikon 9 x 40 binoculars. Each of the ten *G. braunii* plants were observed for four, 10 minute intervals between each of 6 – 9 am, 11 – 2 pm and 3 – 6 pm on two consecutive days, resulting in a total observation time of 240 minutes per observation plant. Each of the eight *A. brunneus* plants were observed for 8 x 10 minute intervals between the same time periods but on just one day, resulting in the same total observation time per plant of 240 minutes. Two *A. djurensis* plants were observed concurrently for four 10 minute intervals between 7 – 8 am, and 3 – 4 pm, whilst another pair was observed immediately after; between 8 – 9 am and 4 – 5 pm. On day two, the time intervals were swapped around so the second pair was observed first during both time intervals. This resulted in a total observation time of 160 minutes

per plant for *A. djurensis*. The length of time (seconds) each bird spent within the focal area was recorded. For *G. braunii* and *A. brunneus*, the number of visits made by birds to individual flowers was also recorded.

All avian flower visitors within the focal area were identified on site using a field guide (Borrow and Demey 2001), whilst insect specimens were photographed using a Canon Power Shot A640 camera and later identified to family level (R. Didham pers. comm.). Footage of sunbirds foraging on *G. braunii* flowers was also obtained using a Canon handheld digital video camera.

Analysis

To test whether time of day had a significant effect on number of flowers visited and time spent within focal area, generalised linear models (GLM's) were run using the statistical program R. The response variables were a) total number of visits (per 80 minute observation period) (*G. braunii* and *A. brunneus* only) and b) total seconds in plant (per 80 minute observation period). Plant was added as a factor into the model before time of day as a block effect to account for the difference between study plants. A quasipoisson error distribution with a log link function was used for all models testing time of day effect, because i) the residual deviance was more than twice that of the residual degrees of freedom (df) indicating overdispersion, ii) the response variables (treated here as count data) were not normally distributed and iii) because it was desirable to restrict the fitted values of the model to greater than ≥ 0 .

For comparisons between mistletoe species, total number of visits made by pollinators and total time spent in the focal area of each plant during each 80 minute observation period were converted to rates per flower per hour by dividing the time spent or number of visits by the number of ripe buds and open flowers within the focal area. This calculation provides an estimate of the attention each flower would likely receive by pollinators per hour, and follows the same methodology of Robertson et al. 1999, 2005 in order to make useful comparison. Visits for *G. braunii* were combined from day one and day two, and visitation rates per 80 minute observation period were calculated using the average number of ripe buds and flowers between the two days.

2.6.4 Role of pollinators

In 2007/08 the role of pollinators was studied for *G. braunii* and *A. brunneus*. At the end of the observation day (or for *G. braunii* at the end of day two) 30 ripe flower buds were selected on each study plant. *G. braunii* buds were recognised as being ripe if the bud tip had darkened, whilst for *A. brunneus* and *A. djurensis* buds, ripe buds were identified by the formation of fenestrae near the apex of the corolla tube. Each bud was individually marked with a numbered paper tag and coloured cotton tie to enable the fate of each individual bud to be followed under different treatments.

The 30 selected buds were divided evenly amongst the following three treatments:

1. Bagged: Ten tagged ripe buds were enclosed inside a 50 cm x 40 cm nylon mesh (0.5mm) bag to exclude all pollinators and ascertain whether buds were capable of a) self-opening and b) autonomous selfing or apomixis resulting in the production of fruit in the absence of pollinators. The bag remained secured until the ovary either aborted or begun swelling
2. Caged: A cage with a mesh size of 2 cm x 2.5 cm was fashioned out of chicken wire and secured around ten tagged ripe buds to exclude avian pollinators but allow insect pollinators access. The cage remained secured until the ovary either aborted or begun swelling. This treatment enabled the comparative role of insect and avian pollinators to be assessed.
3. Natural: Ten ripe buds were tagged and left accessible to all pollinators to measure natural flower opening and fruit set rates.

In the following flowering season of 2008/09, treatments were applied to ten flowering *G. braunii* plants including six of the ten used in 2007/08. Five of the same eight *A. brunneus* plants were also used in 2008/09, with no further flowering plants accessible for study being found. In 2008/09, eight *A. djurensis* plants were also selected for treatment, four of which were those used for pollinator observations.

The same pollination treatment procedure was used as in 2007/08 for all three mistletoe species, except for the inclusion of two new treatments:

4. Hand-selfed: Ten tagged ripe buds were hand-opened and enclosed inside a bag to exclude all pollinators until the ovary either aborted or begun swelling. Pollen from three or more flowers on the same plant was then applied to the stigma of the opened flowers using a fine paint brush to test for self-compatibility. Flowers were left one day before repeating hand-pollination.
5. Hand-crossed: Ten ripe buds were tagged, hand-opened and cross-pollinated using pollen from several flowers on three or more nearby plants to test for pollen limitation. Flowers were left one day before repeating hand-pollination.

In total, in 2007/08 pollination treatments were applied to 300 *G. braunii* flower buds on ten study plants and 240 *A. brunneus* flower buds on eight study plants. In 2008/09, treatments were applied to 500 *G. braunii* flower buds on ten study plants, 250 *A. brunneus* flower buds on five study plants, and 400 *A. djurensis* flower buds on eight study plants.

Following pollination treatment in both seasons, each ripe bud was revisited every few days to monitor its status, i.e. whether it was:

1. A ripe bud
2. Withered bud (still on plant)
3. Open flower stage one (*G. braunii*)
4. Open flower stage two
5. Withered flower (still on plant) stage one
6. Withered flower (still on plant) stage two (*G. braunii*)
7. Ripening fruit (flower has abscised and ovary is swelling)

If the flower bud was observed to contain a Lepidopteran larva ($n=4$), this was excluded from the data for analyses. Once all the ovaries of all flowers had either been aborted or were setting fruit, checks were made weekly and the status of the developing fruit was recorded. A flower was

considered to have successfully set fruit if the developing ovary remained on the plant >8 weeks for *G. braunii*, > 4 weeks for *A. brunneus* and > 5 weeks for *A. djurensis*. These time spans were selected by identifying the development period beyond which the majority of early fruit failures ceased and reflects the variation in fruit development times between species.

After returning to New Zealand, fruit set data continued to be collected at Ngel Nyaki by my field assistant Usman Usuf until all monitored flowers either set fruit or aborted.

For *G. braunii*, a further experiment was carried out to ascertain whether there was a significant time delay in flower opening between ripe buds accessible to pollinators and those under the bagged treatment where pollinators were excluded. In 2008/09, a total of 25 bagged and 32 natural *G. braunii* buds distributed across three different plants were followed daily and the number of days from when the bud first became ripe until when it was first observed open was recorded. Where buds were opened before achieving full ripeness, this was recorded as 0 days in ripe condition.

Analysis

To test whether pollination treatment had a significant effect on a) flower opening and b) fruit set, generalised linear models (GLM's) were run using R. The response variables were a) flower opened or unopened, b) if opened, stage one or stage two pollination (*G. braunii* only) and c) successful fruit set or failure. Where the fate of the bud was unknown, data were not included in the analysis. Due to the outcome of the fate categories being either yes or no, a binomial error distribution with a logit link function was used. The data were treated as a randomized block design with plant entered into each model first as a block effect. Where data were collected during two seasons (*G. braunii* and *A. brunneus*), data were combined and year was added into the model after plant to account for differences between years. An interaction between year and treatment was also included in the initial models to test for differences between years in the effect of treatment on flower opening or fruit set. For all models, significance was tested using a Chi-squared test. All GLM's were followed by a Tukey's honestly significant difference (HSD) multiple comparison test to determine which treatments were significantly different. A pollen limitation index (PLI) was calculated for each mistletoe species using the formula $PLI = 1 - (P_n/P_x)$, where P_n is the percent fruit set of naturally pollinated flowers and P_x is the percent fruit set by plants that received hand-crossed pollen. A PLI of 0 indicates no pollen limitation in the

population studied, whereas a PLI of 1 indicates complete pollen limitation (Larson and Barrett 2000).

To test whether bagging had an effect on number of days a flower bud remained in the ripe condition (for *G. braunii* only) a GLM was run with a poisson error distribution and a log link function. Plant was added as a block effect into the GLM's before treatment. Significance was tested using a Chi-squared test.

2.7 Results

2.7.1 Flower development

Globimetula braunii

In both the 2007/08 and 2008/09 season, flowering of *G. braunii* had commenced by early November and continued for approximately six weeks. The flowering season had peaked by early December, with most plants in early fruit set by late December. Plants were not synchronized in their flowering, with some plants setting fruit whilst others were still developing flower buds. Flower development within individual plants was also asynchronous, with umbels and branches of buds often developing at different times. Flowering of individual plants lasted for approximately 3 weeks. Flowering in 2008/09 appeared to be lower than the previous 2007/08 season with four of the 2007/08 study plants either dead or barely flowering, to the extent they could not be used again in 2008/09.

Darkening of the bud-head from pink to black (signifying ripeness) occurs within 48 hours. On opening, not all flowers were observed to release pollen, and the amount of pollen appeared to vary considerably between flowers and plants. The stigma and style usually appear a pinkish red on opening, often darkening over the next few days. However, I also observed bright green stigmas on pinkish red styles in several freshly opened flowers.

Once opened (either first or second stage), the flower changes colour little aside from the browning and drying of the stigma, anthers and petals over the next 2 – 5 days. The petals remains attached to the ovary for an average of 11 days before either the whole structure abscises

or the petals abscise from the developing ovary. Flower galls were common on plants during flowering (see Appendix 2).

I often observed the formation of very small fenestrae halfway down the mature unopened flower buds with nectar oozing from them. Although this has not been previously recorded for this species, it appears these fenestrae may form as the flower is beginning to ‘self-open’ (Fig 2.9).



Fig 2.9: Ripe *G. braunii* bud thought to be self-opening

A few closed *G. braunii* buds were observed to be infested with a single Lepidopteran larva (Fig 2.10). It appeared that the larva had been growing inside the bud, and ate through the bud to exit on completion of their larval stage. In 2007/08, a total of four flower buds on three study plants were found to contain Lepidopteran larva



Fig 2.10: Lepidopteran larvae inside *G. braunii* bud

Nectar production was measured from 9 – 16 December 2007. Of the 22 flowers measured, 20 (91%) contained nectar in the closed bud on opening, averaging 6.34 μl per flower across the 22 flowers (Table 2.2). The two flowers that contained no nectar on day one were not found to contain nectar on any subsequent days. Eight (36%) flowers contained nectar again on day one having produced on average 1.57 μl per flower across the 22 flowers. No flowers contained nectar after day one, with the majority (81%) of all nectar produced being harvested when the flowers were first opened (Fig. 2.13).

Sugar concentration was measured for all flowers containing sufficient nectar for the refractometer to operate, and was on average 12.8 % on opening and 13.25 % on day one (Table 2.2). Nectar production in *G. braunii* at Ngel Nyaki was found to be higher than has been recorded elsewhere for this species (Table 2.1) (Kirkup 1984). The mean volume of nectar produced per flower over its lifetime was 7.83 μl whilst mean mg sugar per flower over its lifetime was 0.92 mg (Table 2.2).

Agelanthus brunneus

The *Agelanthus brunneus* flowering season commenced in both 2008 and 2009 in mid-January, well into the dry season, and continued through until late February for approximately five weeks. Peak flowering appeared to be in early February. As with *G. braunii*, plants did not flower in synchrony and flowers within individual plants were also unsynchronized. Flowering of individual plants lasted for approximately three weeks. The petals of *A. brunneus* flowers remain attached to the ovary for a long period, some longer than a month, though the majority abscise after 2-3 weeks as estimated from the weekly fruit set checks.

Nectar production was measured from 11-31 January 2008. Twenty-six of 30 (87%) flowers contained nectar on opening, averaging 7.72 μl of nectar per flower across the 30 flowers (Table 2.2). Both the percentage of flowers producing nectar (Table 2.2), and total nectar production (Fig. 2.13) declined in the first four days after opening with just 7% of flowers accounting for 1% of total nectar production by day four. One flower produced nectar on day 8 (5.07 μl) and 9 (0.34 μl) and interestingly, that flower had not produced nectar on day 6 or 7.

The sugar concentration of nectar per flower averaged 19.98 % on opening, with an overall decrease in concentration over the 8 days that flowers continued to produce nectar (Fig. 2.11).

The mean volume of nectar an *A. brunneus* flower produced over its lifetime was 12.16 μl whilst mean mg sugar per flower over its lifetime was 1.30 mg (Table 2.2).

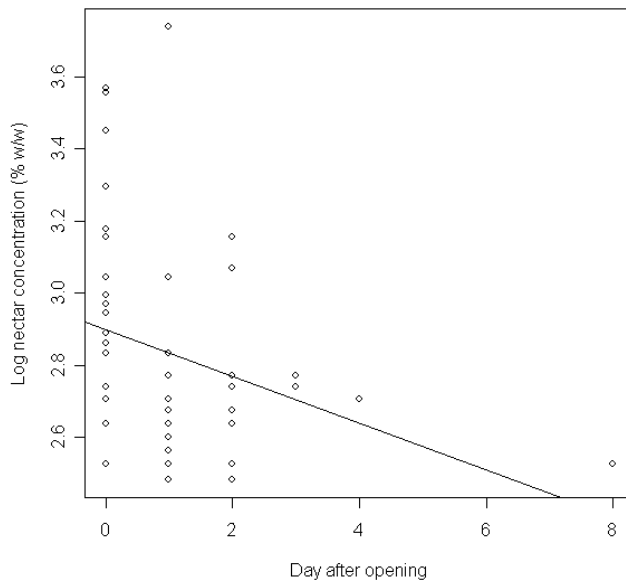


Fig 2.11: Effect of number of days after opening on *A. brunneus* nectar concentration ($F_{1,53} = 5.82$, $p = 0.02$, $y = -0.065x + 2.901$). The regression was still significant when the single value on day 8 was treated as an outlier and removed ($F_{1,52} = 5.01$, $p = 0.03$, $y = -0.086x + 2.916$).

Agelanthus djurensis

Agelanthus djurensis flowering, like *G. braunii*, had commenced by early November 2007 and 2008 and continued for approximately five weeks, peaking late November. Flowering of *A. djurensis* appeared to be more synchronized among plants than was observed for *G. braunii* and *A. brunneus*; however flower development within individual plants was still asynchronous with branches of flowers appearing to develop at different times. Flowering of individual plants lasted approximately three weeks. The entire flower flushes dark pink 2-5 days after opening. I observed colour variations within individual plants whereby bud tips were green through to maturity, or colour banding was completely absent and the whole corolla was light pink throughout development. Flower petals remain attached to the ovary for an average of 14 days before either the whole flower abscises or the petals abscise from the developing ovary.

Nectar production was measured from 21 November to 1 December 2008. Ten of the 11 (91%) flowers contained nectar on opening, containing an average of 6.61 μ l per flower across the 11 flowers (Table 2.2). Over 50% of flowers continued to produce nectar for five days after opening, with no flowers producing nectar after day 7 (Table 2.2). Although nectar quantities were

generally highest on opening, one bud which didn't contain nectar on opening presented nectar (10 μ l) on day two after opening.

Sugar concentration averaged 19.8 % on opening with an overall decrease in concentration over the 7 days that flowers continued to produce nectar (Fig. 2.12).

In contrast to nectar production by *G. braunii* and *A. brunneus*, the decline in nectar production of *A. djurensis* was more gradual, with only 34.8 % of total nectar produced harvested when the buds were first opened (Fig 2.13). The mean volume of nectar a flower produced over its lifetime was 19 μ l whilst mean mg sugar per flower over its lifetime was 0.86 mg (Table 2.2).

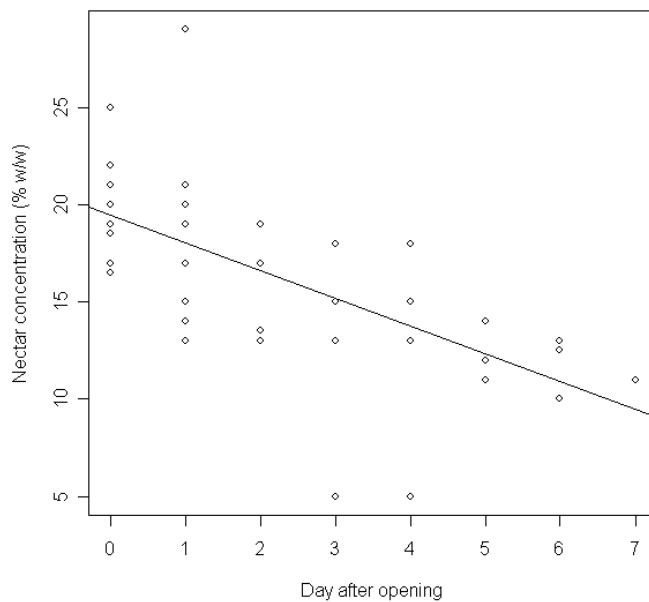


Fig 2.12: Effect of number of days after opening on *A. djurensis* nectar concentration. Regression was highly significant ($F_{1,47} = 35.6$, $p < 0.001$, $y = -1.423x + 19.457$).

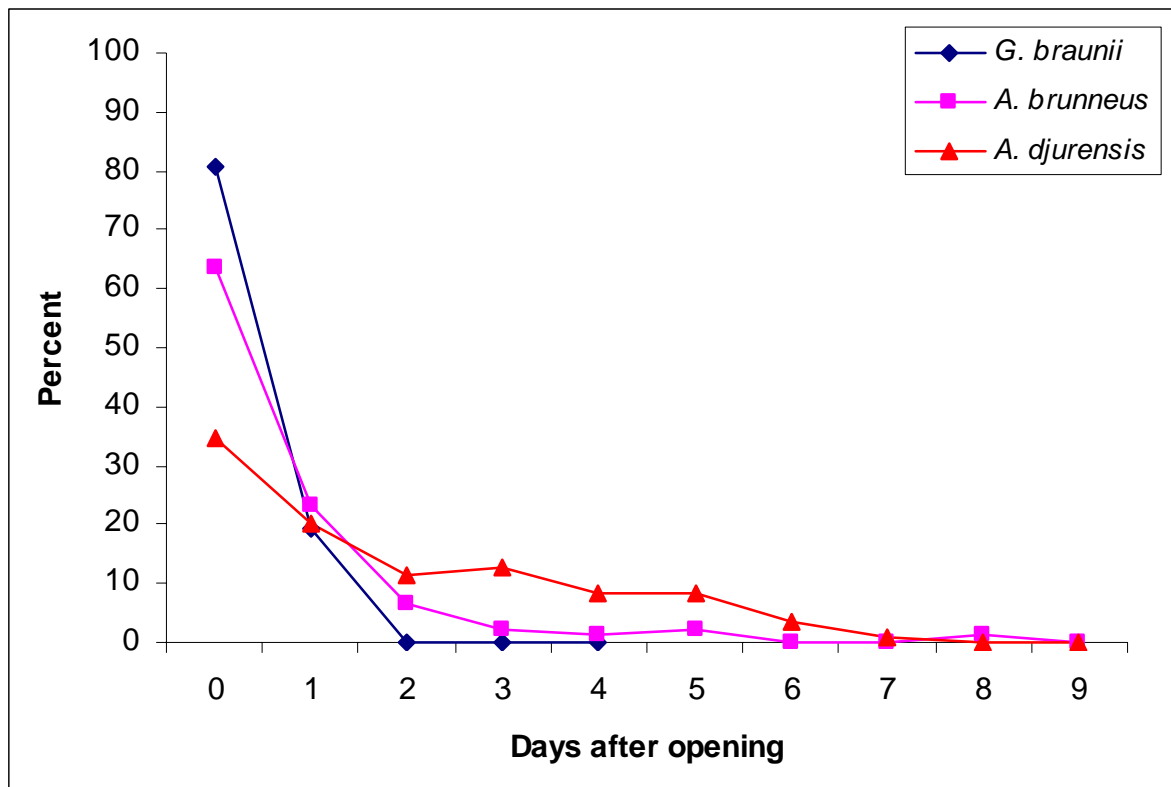


Fig 2.13: Percentage of nectar produced each day for each mistletoe species as a function of total nectar production.

Table 2.2: Summary of nectar production for *Globimetula braunii*, *Agelanthus brunneus* and *Agelanthus djurensis*. The mean volume (μl standard error) of nectar produced for each day after flower opening (day 0) is presented (averaged over all flowers during period where \geq one flower produced nectar i.e. day 0-1 for *G. braunii*, day 0-9 for *A. brunneus* and days 0-7 for *A. djurensis*). The mean sugar concentration (% w/w \pm standard error) is also given with concentrations averaged for n flowers where concentration was obtained. Total mean volume (μl \pm standard error) nectar and total mean mg sugar produced over life of flower is also presented. Dash (–) indicates no flowers measured.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Vol (μl) per flower	Sugar (mg) per flower
<i>G. braunii</i> (n=22)											7.83 \pm 1.19	0.92 \pm 0.13
Proportion of flowers with nectar	91%	36%	0	0	0	–	–	–	–	–		
Volume μl	6.34 \pm 0.91	1.57 \pm 0.68	0 \pm 0	0 \pm 0	0 \pm 0	–	–	–	–	–		
Sugar % (w/w)	12.80 \pm 0.66 (n=20)	13.25 \pm 0.44 (n=8)	n/a	n/a	n/a	–	–	–	–	–		
<i>A. brunneus</i> (n=30)											12.16 \pm 1.30	1.30 \pm 0.12 (n=28)
Proportion of flowers with nectar	87%	63%	33%	17%	7%	13%	0%	0%	3%	3%		
Volume μl	7.72 \pm 1.06	2.82 \pm 0.54	0.80 \pm 0.30	0.25 \pm 0.14	0.15 \pm 0.13	0.25 \pm 0.13	0 \pm 0	0 \pm 0	0.17 \pm 0.17	0.01 \pm 0.01		
Sugar % (w/w)	19.98 \pm 1.26 (n=25)	16.38 \pm 1.68 (n=17)	16.17 \pm 1.30 (n=9)	15.75 \pm 0.25 (n=2)	15.0 (n=1)	n/a	n/a	n/a	12.50 (n=1)	n/a		
<i>A. djurensis</i> (n=11)											19.00 \pm 0.66	0.86 \pm 0.07
Proportion of flowers with nectar	91%	91%	55%	82%	64%	64%	46%	9%	0%	0%		
Volume μl	6.61 \pm 0.75	3.81 \pm 0.63	2.20 \pm 1.00	2.40 \pm 0.69	1.55 \pm 0.42	1.60 \pm 0.48	0.67 \pm 0.31	0.18 \pm 0.18	0 \pm 0	0 \pm 0		
Sugar % (w/w)	19.80 \pm 0.78 (n=10)	18.70 \pm 1.44 (n=10)	15.63 \pm 1.43 (n=4)	13.63 \pm 1.35 (n=8)	15.33 \pm 0.72 (n=6)	13.0 \pm 0.63 (n=5)	11.83 \pm 0.93 (n=3)	11.0 (n=1)	n/a	n/a		

2.7.2 Pollinator visitation observations

Four species of sunbirds from two genera were observed visiting mistletoe flowers; these were the western olive sunbird (*Cyanomitra obscura*) (Fig 2.14), green headed sunbird (*Cyanomitra verticalis*) (Fig 2.15), northern double collared sunbird (*Cinnyris reichenowi*) (Fig 2.16) and variable sunbird (*Cinnyris venustus*) (Fig 2.17). Honey bees (*Apis mellifera*) and a small social wasp species (Subfamily: Vespinae) were also observed to visit the flowers of *A. brunneus* and *A. djurensis* respectively (Table 2.3).



Fig. 2.14: Western olive sunbird (*C. obscura*)



Fig. 2.15: Green-headed sunbird (*C. verticalis*).
Photo: Tom Tarrant



Fig. 2.16: Male northern-double coloured sunbird (*C. reichenowi*)



Fig. 2.17: Male variable sunbird (*C. venustus*) Photo: Kyle Luman

Table 2.3: Flower visitors observed at each of the three mistletoe plants *Globimetula braunni* (n=10), *Agelanthus brunneus* (n=8) and *Agelanthus djurensis* (n=4). √ = observed, X = not observed

Flower visitor	Size	<i>G. braunni</i>	<i>A. brunneus</i>	<i>A. djurensis</i>
<i>Cyanomitra obscura</i>	13-15 cm	√	√	√
<i>Cyanomitra verticalis</i>	13-14 cm	√	X	√
<i>Cinnyris reichenowi</i>	11.5 cm	√	√	√
<i>Cinnyris venustus</i>	10 cm	√	√	X
<i>Apis mellifera</i>	1.3 cm	X	√	X
Vespinae sp.	0.8-1 cm	X	X	√

Globimetula braunni

Of the ten *G. braunni* plants observed, the focal area contained a mean of 469 ripe buds/open flowers (range 214 – 569). During 40 hours of observations I observed all four species of sunbirds visiting the flowers of *G. braunni*. The most commonly observed sunbird was *C. reichenowi* (Table 2.4) (Fig 2.18). One of the 10 mistletoe plants did not receive any visits at all throughout 240 minutes of observation.

Table 2.4: Flower visitors observed within focal area of ten *G. braunni* plants during 40 hours of observations. Mean number of flowers visited and mean time spent is the mean per each of three 80 minute time intervals.

	Plants visited	Total visits to plants	Mean visits per plant	Mean flowers visited	Mean time spent (seconds)
<i>C. obscura</i>	5 (50%)	13	1.30 ± 0.62	22.7 ± 10.55	73.10 ± 30.22
<i>C. verticalis</i>	1 (10%)	3	0.30	3.90 ± 3.90	17.60
<i>C. reichenowi</i>	6 (60%)	14	1.40 ± 0.76	15.10 ± 7.63	37.40 ± 17.25
<i>C. venustus</i>	1 (10%)	5	0.50	4.20 ± 4.20	26.30

For the nine *G. braunni* plants that were visited at least once by sunbirds, analysis revealed no significant time of day effect on either the length of time sunbirds spent foraging or average number of flowers visited within the focal area (Table 2.5, Fig. 2.19).



Fig 2.18: *C. reichenowi* foraging on *G. braunii* flowers amongst pollination treatments

Table 2.5: Results from the GLM ANOVA models comparing (a) average number of visits within focal area and (b) average time spent (seconds) within focal area at different times of the day across nine *Globimetula braunii* plants in 2007/08. A quasipoisson error distribution was used with an F test for significance.

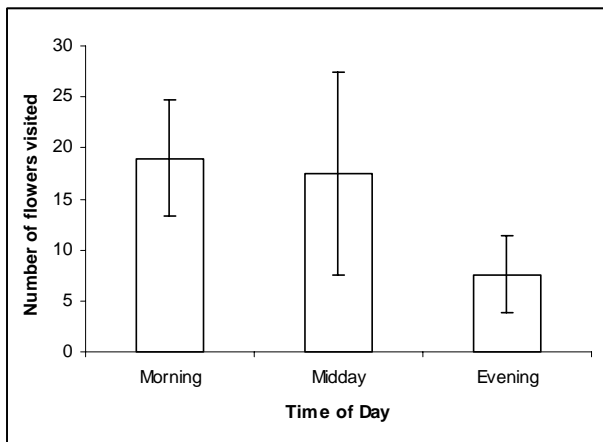
(a)

Factor	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(F)
Null			26	617.000		
Plant (as factor)	8	290.060	18	326.940	2.298	0.075
Time of day	2	52.780	16	274.150	1.673	0.219

(b)

Factor	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(F)
Null			26	2159.550		
Plant (as factor)	8	774.630	18	1384.920	1.203	0.357
Time of day	2	66.100	16	1318.820	0.411	0.670

(a)



(b)

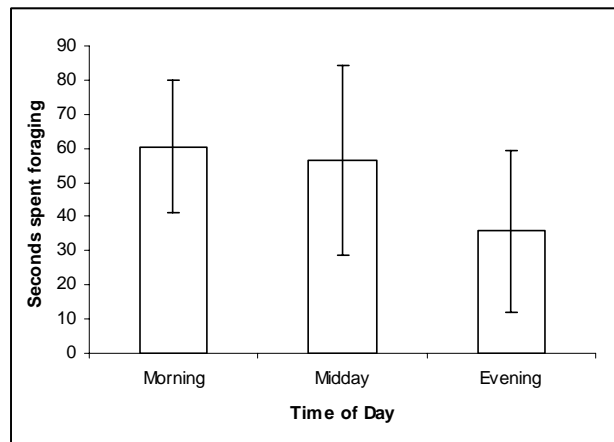


Fig 2.19. Sunbird visitation during different times of the day at nine *Globimetula braunii* plants showing (a) number of flowers visited within focal area of plant and (b) seconds spent foraging within focal area of plants. Means and standard errors are presented. ANOVA's showed no significant difference among times, see Table 2.5.

Agelanthus brunneus

Of the eight *A. brunneus* plants observed, the focal area contained a mean of 375 ripe buds/open flowers (range 119 – 809). In the 32 hours I spent observing *A. brunneus*, I observed three species of sunbird and one species of honey bee visiting the flowers (Table 2.6). The sunbird species observed were the same as those visiting *G. braunii*, with the exception of the Green-headed sunbird *C. verticalis* which was not observed to visit *A. brunneus*. *C. reichenowi* was observed to be the most frequent and consistent flower visitor (Table 2.6). Actual flower visits

and time spent within the focal area were not recorded for the honey bees because an observation station at closer proximity to the focal plant would be required to monitor the bees and this observer distance would deter bird visits.

Table 2.6: Flower visitors observed within focal area of eight *A. brunneus* plants during 32 hours of observations. Mean number of flowers visited and mean time spent is the mean per each of three 80 minute time intervals. Dash ‘—’ indicates no data collected.

	Plants visited	Total visits to plants	Mean visits per plant	Mean flowers visited	Mean time spent (seconds)
<i>C. obscura</i>	1 (13%)	6	0.75	28.13	69.25
<i>C. reichenowi</i>	7 (88%)	20	2.50 ± 0.73	15.88 ± 5.55	65.50 ± 23.56
<i>C. venustus</i>	1 (13%)	1	0.13	0.25	1.00
<i>A. mellifera</i>	2 (25%)	—	—	—	—

One of the two *A. brunneus* plants visited by *Apis mellifera* was observed to have several honeybees present on flowers at any one time (Fig 2.20). *A. mellifera* appeared to be robbing nectar from the flowers, crawling over unopened buds and settling in one place, generally at the base of the corolla, whilst presumably harvesting nectar. The bees rarely contacted the stigmas of the open flowers, though did so occasionally whilst crawling over or flying between flowers. Small perforations were observed all over the corollas of flowers, but most commonly at the base. It is most likely *A. mellifera* did not cause the perforations but instead behaved as ‘secondary nectar robbers’; utilising the perforations as a means of accessing nectar (Inouye 1980).



Fig 2.20: Honey bee *Apis mellifera* robbing nectar from *A. brunneus* flowers. Small perforations at bases of corollas are also visible.

On the plant visited only by *A. mellifera* 152 buds were randomly selected and assessed for damage. A total of 146 (96 %) were damaged with perforations. Ten undamaged immature buds were subsequently bagged and monitored through to flower abscission. None developed perforations suggesting that animals were responsible. Nectar was measured in 30 of these damaged closed buds and none contained any nectar at all. However, all 10 of the bagged undamaged buds contained nectar (mean 10.11 µl) when opened. Interestingly sunbirds were

never observed visiting that plant. Two of the other seven *A. brunneus* plants were also noted to have a significant number of perforations on the flowers, one of which *A. mellifera* was also observed robbing nectar from.

Analysis revealed that of the eight *A. brunneus* plants that were visited by sunbirds, there was no significant difference by time of the day in average number of flowers visited and time spent foraging within the focal area. Differences between plants were highly significant (Table 2.7, Fig. 2.21).

Table 2.7: Results from the GLM ANOVA models comparing (a) average number of visits within focal area and (b) average time spent (seconds) within focal area at different times of the day across eight *Agelanthus brunneus* plants in 2007/08. A quasipoisson error distribution was used with an F test for significance.

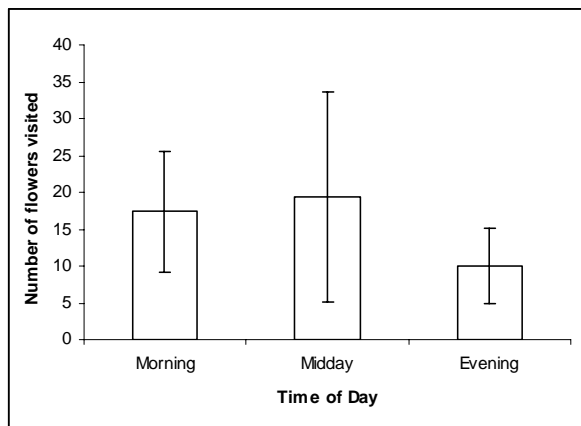
(a)

Factor	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(F)
Null			23	747.310		
Plant (as factor)	7	622.060	16	125.250	12.470	P < 0.001
Time of day	2	26.790	14	98.460	1.880	0.189

(b)

Factor	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(F)
Null			23	1877.860		
Plant (as factor)	7	1457.630	16	420.230	8.599	P < 0.001
Time of day	2	93.440	14	326.790	1.929	0.182

(a)



(b)

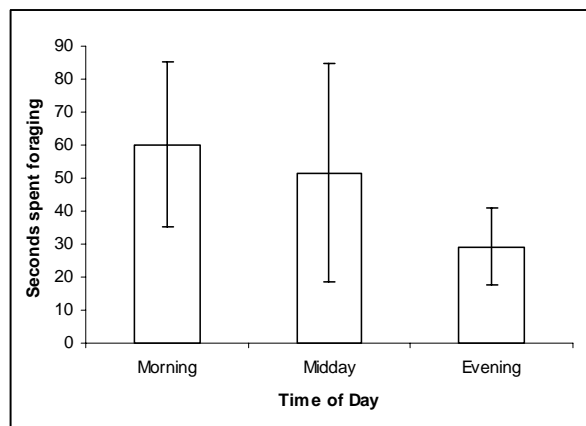


Fig 2.21: Sunbird visitation during different times of the day at eight *Agelanthus brunneus* plants showing (a) number of flowers visited within focal area of plant and (b) seconds spent foraging within focal area of plants. Means and standard errors are presented. ANOVA's showed no significant difference among times, see Table 2.7.

Agelanthus djurensis

Of the four *A. djurensis* plants observed, the focal area contained a mean of 180 ripe buds/open flowers (range 104 – 299). I observed *A. djurensis* for a total of 10.67 hours and observed three species of sunbirds and one wasp species visiting the flowers (Table 2.8). Of the four observation plants, one was not visited at all by sunbirds. All four *A. djurensis* observation plants were checked for visitation by the Vespinae wasp species and all were observed being visited. The additional four plants used in the pollination treatment study were also checked and found to be visited by the wasps. Actual flower visits and time spent within the focal area were not recorded for wasps (see above).

Table 2.8: Flower visitors observed within focal area of four *Agelanthus djurensis* plants during 32 hours of observations. Mean time spent is the mean seconds spent within focal area during each of three 80 minute time intervals. Dash ‘–’ indicates no data collected.

	Plants visited	Total visits to plants	Mean visits per plant	Mean time spent (seconds)
<i>C. obscura</i>	3 (75%)	5	1.25 ± 0.63	68.75 ± 47.71
<i>C. verticalis</i>	1 (25%)	1	0.25	17.00
<i>C. reichenowi</i>	2 (50%)	2	0.50 ± 0.29	11.50 ± 7.40
Vespinae sp.	4 (100%)	–	–	–

Closer investigation of the wasps behaviour on *A. djurensis* flowers revealed that they were harvesting pollen from unopened flowers by curling their body around the flower tip and prying open the corolla lobes with their legs and mandibles, just enough to clean the newly exposed anthers of their pollen (Fig 2.22).



Fig 2.22: Social wasp species (subfamily Vespinae) opening an *A. djurensis* flower

Of the three *A. djurensis* plants that were visited by sunbirds, analysis revealed no significant difference in the length of time sunbirds were observed foraging within the focal area between morning and evening (Table 2.9, Fig. 2.23).

Table 2.9: Results from the GLM ANOVA model comparing length of time sunbirds spent foraging within the focal area of three *Agelanthus djurensis* plants at two different times of the day (morning and evening) in 2008/09. A quasipoisson error distribution was used with an F test for significance.

Factor	Df	Deviance	Residual Df	Residual Deviance	F	Pr(F)
Null			11	430.64		
Plant	2	82.26	9	348.37	1.645	0.252
(as factor)						
Time of day	1	109.06	8	239.31	4.361	0.070

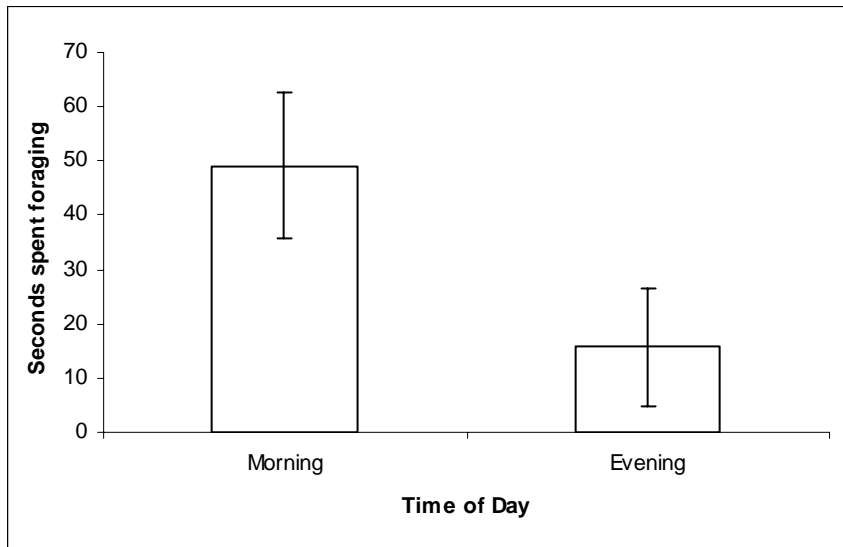


Fig 2.23: Time (seconds) sunbirds spent foraging within the focal area of three *Agelanthus djurensis* plants at two different times of the day. Means and standard errors are presented. ANOVA showed no significant difference among times, see Table 2.9.

On all three mistletoe species, the sunbirds moved quickly through the plant, perching on small branches before inserting their long curved beak into the flowers. It could not be determined whether all four sunbird species were opening the flowers, though only the largest two sunbird species *C. obscura* and *C. verticalis* were actually observed flower opening. *C. obscura* was also frequently observed with large amounts of white pollen on its crown feathers. Already-opened flowers were the most commonly observed to be visited by all sunbird species. A review of handheld video footage showed that sunbird visits to *G. braunni* flowers were between 1-3 seconds in duration (see CD in Appendix 3).

A comparison of sunbird visitation rates reveals that the sunbirds foraged at approximately the same rate at all three mistletoe species (Table 2.10).

Table 2.10: Sunbird visitation rates to flowers of the mistletoes *Globimetula braunni*, *Agelanthus brunneus* and *Agelanthus djurensis*. Dash ‘-’ indicates number of visits not recorded for *A. djurensis*.

Sunbird Visitation Rates					
	(secs/flwr/hr)	S.E	(visits/flwr/hr)	S.E	<i>n</i>
<i>G. braunni</i>	0.107	0.025	0.034	0.009	10
<i>A. brunneus</i>	0.101	0.028	0.033	0.011	8
<i>A. djurensis</i>	0.125	0.042	-	-	4

From these calculated visitation rates, where the lifespan of the flower and the actual duration of a flower visit is known, the total number of visits a flower receives throughout its lifetime can be estimated. Below is a worked example for a *Globimetula braunii* flower:

- Mean flower life = 11 days
- Total available pollination time per day \approx 12 hours per day
- Total available pollination time per flower = 11 days x 12 hrs per day = 132 hours
- Calculated mean visitation time per flower per hour = 0.107 (secs/flwr/hr)
- Total visitation time over each flowers lifespan = 0.107 secs x 132 hrs = 14 seconds

Therefore, if one flower visit takes approximately two seconds (as determined from video footage – see CD enclosed in Appendix 3), each *G. braunni* flower can expect to be visited approximately 7 times during its life time ($14 \div 2 = 7$).

2.7.3 Flower opening and fruit set

Globimetula braunii

Flower opening

In 2007/08, of the 300 flower buds selected for the flower opening experiment, the fate of 272 buds was successfully recorded. In 2008/09, one of 10 study plants selected for the flower opening experiment died, leaving 270 flower buds on 9 study plants for monitoring. Of these 270, the fate of 263 was successfully recorded.

There was a highly significant treatment effect on flower opening (opening to stage one or two) after accounting for differences between study plants and between years (Table 2.11). There was no significant interaction effect between year and treatment (Appendix 1) revealing that the effect of treatment on flower opening did not vary significantly between years.

Table 2.11: Results from the GLM ANOVA model testing *G. braunii* flower opening under bagged, caged and natural pollination treatments across thirteen plants (n=535 flowers) in 2007/08 and 2008/09. A binomial error distribution with a chi-squared test for significance was used.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			56	240.45	
Plant	12	55.974	44	184.48	P < 0.001
Year	1	31.171	43	153.31	P < 0.001
Treatment	2	79.437	41	73.87	P < 0.001

Flowers under natural conditions opened 95.5% of the time on average, which was the highest proportion of opening under any of the three treatments (Fig 2.24). Flower opening under the bagged and caged treatment was 66.6% and 66.1% respectively, suggesting that the flowers of *G. braunii* are able to self-open in the absence of bird pollinators, but to a lesser extent than is achieved when bird pollinators have access.

There was no significant difference in the proportion of flowers opened between the bagged and caged treatment (Tukey HSD; $p > 0.05$) indicating that insects do not contribute significantly to flower opening in *G. braunii* (Table 2.12).

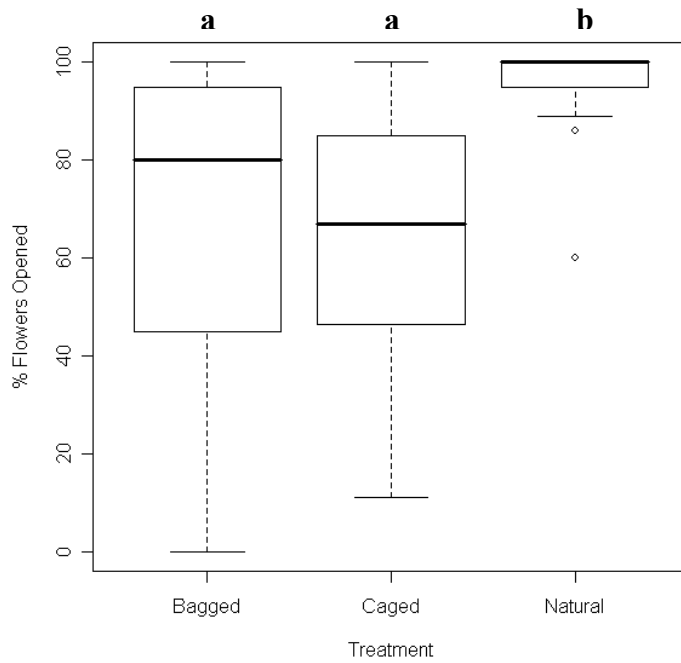


Figure 2.24: Percentage of *G. braunii* flowers opened under three pollination treatments in 2007/08 and 2008/09. Circles represent outliers. Each box shows median percentage of flowers opened and the 95th, 75th, 25th and 5th percentiles. Total number of flowers opened = 408 of 535 (76%). Results from Tukey's HSD test are also indicated as letters above boxes.

Table 2.12: Results from Tukey's HSD multiple comparison testing *G. braunii* flower opening under different pollination treatments. Shown are 95% confidence intervals and respective P values.

Treatments compared	Difference	Lower CI	Upper CI	P
Caged vs. Bagged	-0.53	-17.59	16.54	0.997
Natural vs. Bagged	28.84	11.78	45.90	< 0.001
Natural vs. Caged	29.37	12.30	46.43	< 0.001

Analysis revealed a significant treatment effect on the number of flowers reaching stage two pollination once they are already open to at least stage one, after accounting for significant variability between study plants and years (Table 2.13). Once again, there was no significant interaction effect between year and treatment indicating that the effect of treatment on a flower reaching second-stage pollination did not vary significantly between years (Appendix 1).

Table 2.13: Results from the binomial GLM ANOVA model using Chi squared analysis to compare the number of open *G. braunii* flowers which reached stage one versus stage two pollination under bagged, caged and natural pollination treatments across thirteen plants (n=408 flowers) in 2007/08 and 2008/09.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			55	128.232	
Plant	12	26.227	43	102.004	0.010
Year	1	21.542	42	80.463	P < 0.001
Treatment	2	34.382	40	46.080	P < 0.001

On average 97.8% of open flowers reached stage two pollination under natural conditions (accessible to all pollinators), compared with 72.6% and 81% under the bagged and caged conditions respectively (Fig. 2.25). A Tukey's HSD showed that a significantly higher proportion of flowers under natural conditions achieved second stage pollination when compared to those inside a bag, suggesting that birds had a significant effect on whether or not a flower reached second stage opening if already open (Table 2.14).

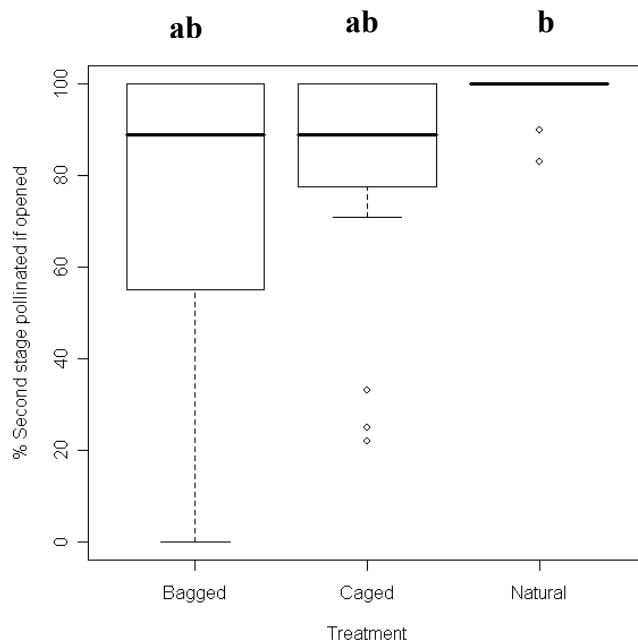


Figure 2.25: Percentage of opened *G. braunii* flowers that achieved second stage pollination under three pollination treatments in 2007/08 and 2008/09. Circles represent outliers. Each box shows median percentage of flowers opened and the 95th, 75th, 25th and 5th percentiles. Total number of flowers that achieved stage two opening = 366 of 408 (90%). Results from Tukey's HSD test are also indicated as letters above boxes.

Table 2.14: Results from Tukey's HSD multiple comparison tests comparing percentage of *G. braunii* flowers that achieved second-stage flower opening between pollination treatments. Shown are 95% confidence intervals and respective P values.

Treatments compared	Difference	Lower CI	Upper CI	P
Caged vs. Bagged	8.42	-11.54	28.39	0.566
Natural vs. Bagged	25.11	5.14	45.07	0.011
Natural vs. Caged	16.68	-3.28	36.65	0.118

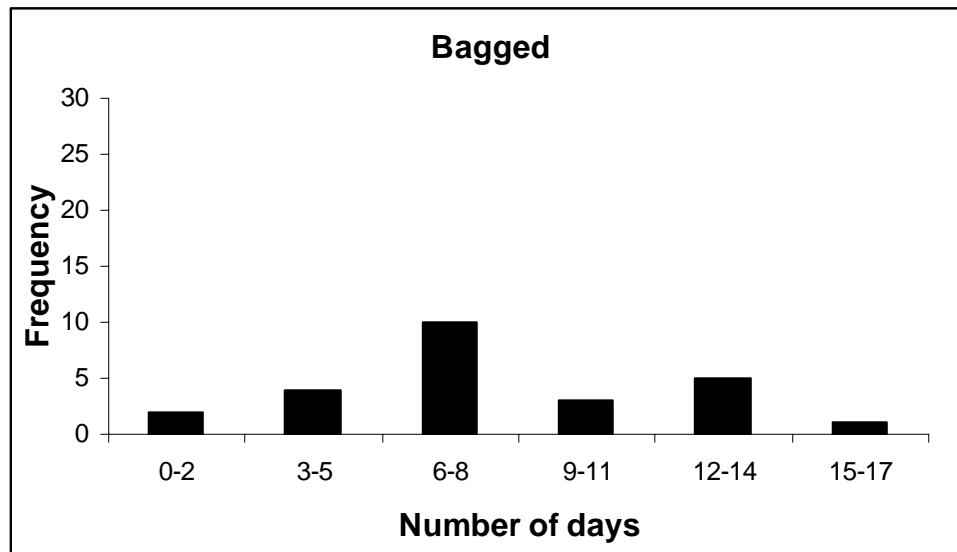
Overall, these results reveal that the flower buds of *G. braunii* are capable of self-opening, however sunbirds appear to significantly increase flower opening and also ensure that open flowers achieve opening to second stage.

The number of days a ripe *G. braunii* bud took to open was significantly longer under bagged conditions than natural conditions (Table 2.15). When buds were enclosed within a bag to exclude all pollinators, ripe buds remained unopened for 7.9 days on average before self-opening, with the mode between 6 – 8 days (Fig 2.26). The first bud to open after achieving ripeness in the bag was on day two, and the longest a bagged bud remained ripe before opening was 15 days. In contrast, under natural conditions ripe buds took on average 1.6 days to open, with 4 buds open before they were fully ripe (achieving 0 days of ripeness), 15 open the day after being first observed ripe and a further 8 open on day two (Fig. 2.26) The last ripe buds to be opened under natural conditions were opened on Day 6.

Table 2.15: Results from the poisson GLM ANOVA model using Chi squared analysis to compare the number of days that ripe *G. braunii* buds remained unopened under bagged and natural treatments across 4 plants (n=57) in 2008/09.

Factor	Df	Deviance	Residual Df	Residual Deviance	Pr(Chi)
Null			56	205.34	
Plant	3	8.41	53	196.94	0.038
Treatment	1	126.63	52	70.31	P < 0.001

(a)



(b)

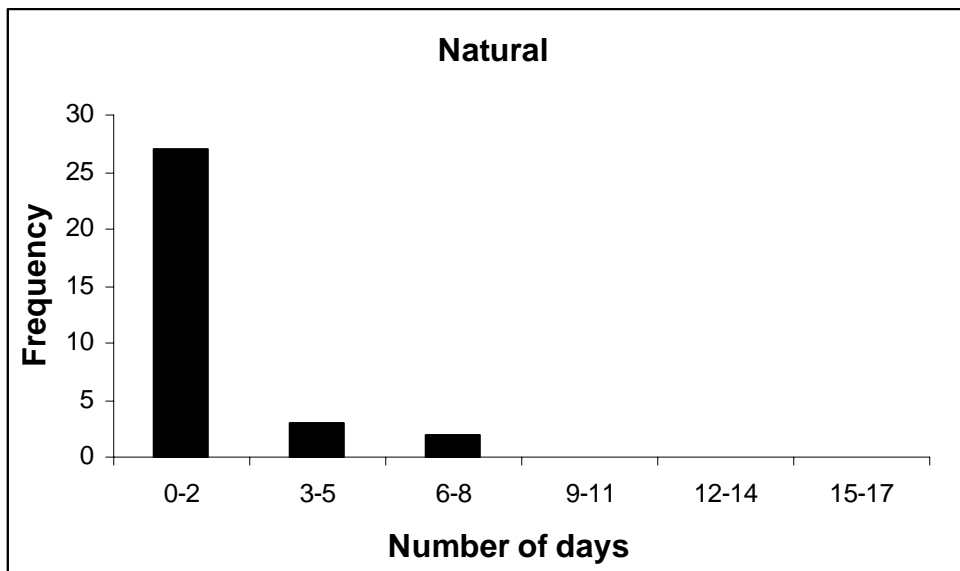


Figure 2.26: Bar chart showing the number of *G. braunii* buds opening each day after first being recorded as ripe under (a) bagged (n = 25) and (b) natural (n = 32) treatments.

Fruit set

In 2007/08, only 22 of the 300 *G. braunii* flowers produced fruit, resulting in an overall mean fruit set of 7% across the three pollination treatments. In 2008/09, when two additional hand-pollination treatments were applied, a total of 156 of 450 (35%) flowers set fruit across the five treatments.

Analysis confirmed a highly significant difference in fruit set between pollination treatments, with treatment group explaining the majority of the variance in fruit set (Table 2.16). There was no significant interaction effect between year and treatment (Appendix 1) revealing that the effect of treatment on fruit set did not vary significantly between years.

Table 2.16: Results from the GLM ANOVA model comparing *G. braunii* fruit set under bagged, caged, natural, crossed and selfed pollination treatments across thirteen plants (n=750 flowers) in 2007/08 and 2008/09. Crossed and selfed treatments were only applied in 2008/09. A binomial error distribution was used with a chi-squared test for significance.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			74	386.52	
Plant	12	40.63	62	345.89	P < 0.001
Year	1	70.84	61	275.05	P < 0.001
Treatment	4	200.44	57	74.61	P < 0.001

No *G. braunii* flowers set fruit inside bags, 7.4% set fruit under caged conditions and 27.4% under natural conditions (Fig 2.27). Tukey's HSD tests confirm that there was no significant difference in fruit set between the bagged and caged treatments (Table 2.17). These results indicate that insects do not play a significant role in the fruit set of *G. braunii*.

There was no significant difference in mean fruit set between hand-crossed (54.4%) and hand-selfed flowers (70%) (Table 2.17). High fruit set amongst hand-selfed flowers indicated that *G. braunii* is highly self-compatible. However, despite on average 66% of flowers self-opening explosively, the fact that no flowers under the bagged condition produced fruits in either season clearly indicates that *G. braunii* flowers are not autonomous. Hand-pollinated flowers had higher fruit set than those under natural conditions indicating that in the years studied, *G. braunii* was pollen limited (PLI = 0.504) (Fig 2.27).

Overall, natural fruit production in *G. braunii* was almost entirely reliant on sunbirds in both 2007/08 and 2008/09, and the sunbirds did not appear to be providing adequate service to meet the reproductive potential of this species.

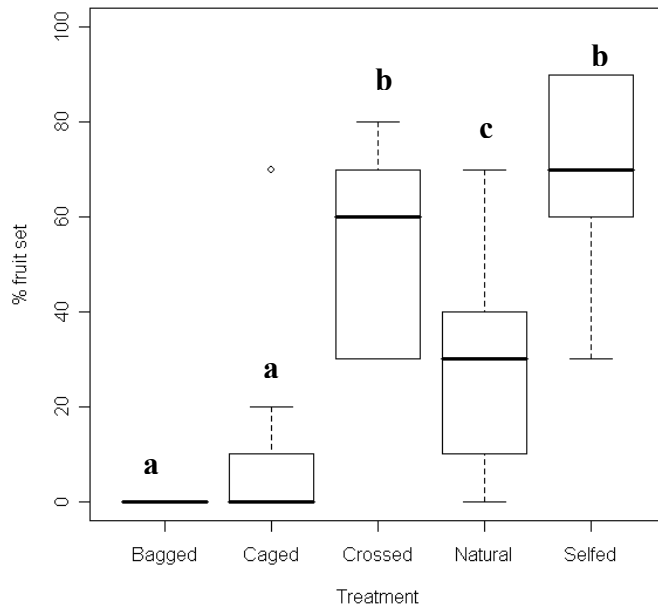


Figure 2.27: Percentage of *G. braunii* flowers that set fruit under different pollination treatments in 2007/08 and 2008/09 across twelve study plants. Crossed and selfed treatments were only applied in 2008/09. Each box shows median percentage of flowers that produced fruit and the 95th, 75th, 25th and 5th percentiles. Circle represents an outlier. n= 750. Results from Tukey's HSD test are also indicated as letters above boxes.

Table 2.17: Results from Tukey's HSD multiple comparison tests comparing *G. braunii* fruit set between different pollination treatments. Shown are 95% confidence intervals and respective P values.

Treatments compared	Difference	Lower CI	Upper CI	P
Caged vs. Bagged	7.37	-7.79	22.53	0.650
Crossed vs. Bagged	50.51	31.60	69.42	P < 0.001
Natural vs. Bagged	27.37	12.21	42.53	P < 0.001
Selfed vs. Bagged	66.07	47.16	84.98	P < 0.001
Crossed vs. Caged	43.14	24.23	62.05	P < 0.001
Natural vs. Caged	20.00	4.84	35.16	0.004
Selfed vs. Caged	58.70	39.79	77.61	P < 0.001
Natural vs. Crossed	-23.14	-42.05	-4.23	0.009
Selfed vs. Crossed	15.56	-6.47	37.59	0.285
Selfed vs. Natural	38.70	19.79	57.61	P < 0.001

Agelanthus brunneus

Flower opening

In 2007/08, 240 *A. brunneus* flower buds were used in the flower opening experiment and the fate of 222 buds was successfully recorded. In 2007/08, the fate of all 150 flower buds used in the flower opening experiment was recorded.

A comparison of flower opening between treatments revealed a highly significant treatment effect on flower opening (Table 2.18). Treatment effect varied significantly between years as shown by the interaction effect between treatment and year.

Table 2.18: Results from the GLM ANOVA model testing *A. brunneus* flower opening under bagged, caged and natural pollination treatments across eight plants (n=372 flowers) in 2007/08 and 2008/09. A binomial error distribution was used with a chi-squared test for significance.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			38	322.98	
Plant	7	46.06	31	276.92	P < 0.001
Year	1	32.90	30	244.02	P < 0.001
Treatment	2	182.01	28	62.01	P < 0.001
Year:Treatment	2	21.63	26	40.38	P < 0.001

Only 1.3% bagged buds opened in 2007/08 and none in 2008/09 (Fig. 2.28). These results reveal that the flowers of *A. brunneus* do not self-open. In 2007/08, on average 27.9% of flowers under natural conditions were opened compared with 100% in 2008/09.

Tukey's HSD tests showed that the proportion of flowers opened inside a cage (38.3%, 2007/08; 82%, 2008/09) was not significantly different from those under natural conditions in either season (Table 2.19). This indicates that insects are capable of opening the flowers of *A. brunneus*, and that both sunbirds and insects are equally as effective in opening the flowers of this species.

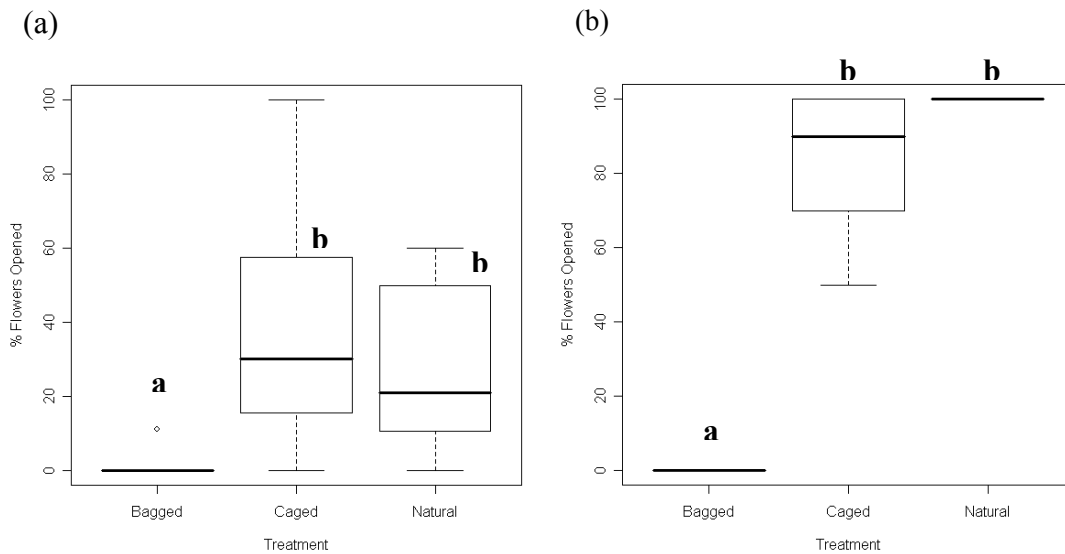


Figure 2.28: Percentage of *A. brunneus* flowers opened under three pollination treatments in (a) 2007/08 and (b) 2008/09. Circle represents an outlier. Each box shows median percentage of flowers opened and the 95th, 75th, 25th and 5th percentiles. (a) number of flowers opened = 48 of 222 (22%) (b) number of flowers opened = 91 of 150 (61%). Results from Tukey's HSD test are also indicated as letters above boxes.

Table 2.19: Results from Tukey's HSD multiple comparison testing *A. brunneus* flower opening under different pollination treatments. Shown are 95% confidence intervals and respective P values.

Year	Treatments compared	Difference	Lower CI	Upper CI	P
2007/08	Caged vs. Bagged	36.88	12.83	60.92	0.003
	Natural vs. Bagged	26.50	2.45	50.55	0.030
	Natural vs. Caged	-10.38	-34.42	13.67	0.513
2008/09	Caged vs. Bagged	82	59.38	104.62	P < 0.001
	Natural vs. Bagged	100	77.38	122.62	P < 0.001
	Natural vs. Caged	18	-4.62	40.62	0.118

Fruit set

In 2007/08, of the 240 *A. brunneus* flowers selected for treatment, 14 (6%) set fruit across the three pollination treatments. In 2008/09, 54 of 250 flowers across five pollination treatments set fruit, resulting in an average of 22.6% fruit set overall.

Analysis revealed fruit set was significantly different between study plants, year and treatments, with treatment effect varying significantly between years (Table 2.20).

Table 2.20: Results from the GLM ANOVA model comparing *A. brunneus* fruit set under bagged, caged, selfed, natural and crossed pollination treatments across eight plants in 2007/08 and 2008/09.

A binomial error distribution was used with a chi-squared test for significance.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			48	149.15	
Plant	7	21.76	41	127.39	0.003
Year	1	15.69	40	111.73	P < 0.001
Treatment	4	32.74	36	78.99	P < 0.001
Year:Treatment	2	38.10	34	40.89	P < 0.001

Fruit set under natural conditions was higher in 2008/09 (40%) than in the 2007/08 (2.5%) (Fig 2.29). Flowers almost never set fruit within the bags (5%, 2007/08; 0%, 2008/09) indicating that *A. brunneus* is not autonomous. Fruit set within the cages was low (10%, 2007/08; 0%, 2008/09) and not significantly different from fruit set within a bag in either year indicating that insects do not play a significant role in fruit set of *A. brunneus*. Hand-selfed flowers produced on average 32% fruit set revealing that *A. brunneus* is highly self-compatible. A Tukey's HSD showed no significant difference in fruit production between the hand-crossed (36%), natural and hand-selfed pollination treatments (Table 2.21). This indicates that in 2008/09, *A. brunneus* was not pollen limited (PLI = 0).

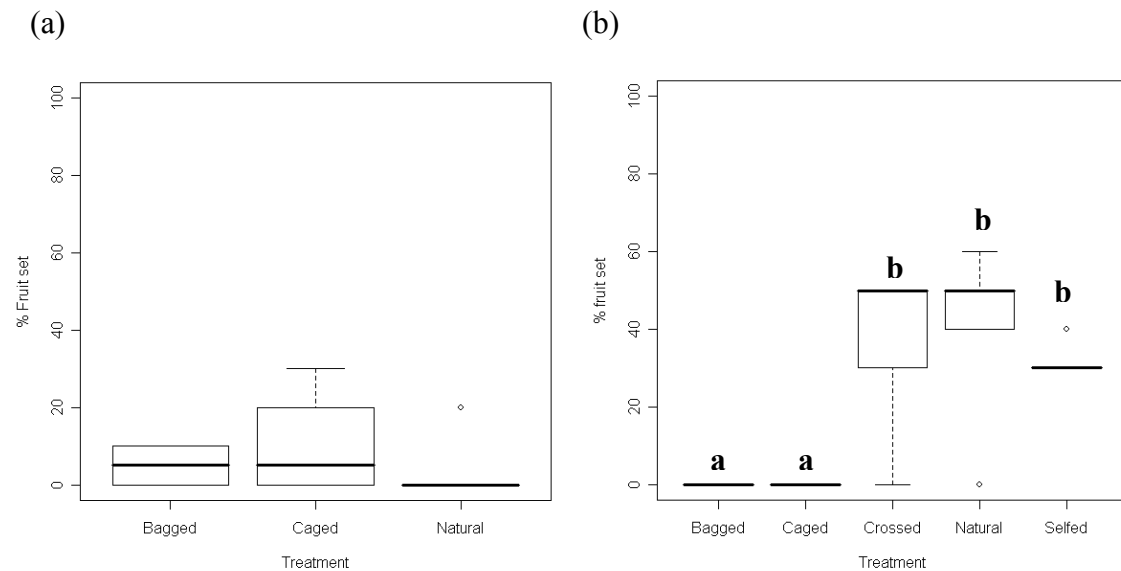


Figure 2.29: Percentage of *A. brunneus* flowers that set fruit under different pollination treatments in a) 2007/08 across eight study plants (n=240) and b) 2008/09 across five study plants (n=250). Each box shows median percentage of flowers that produced fruit and the 95th, 75th, 25th and 5th percentiles. Circles represent outliers. Results from Tukey's HSD test are also indicated as letters above boxes.

Table 2.21: Results from Tukey's HSD multiple comparison test comparing *A. brunneus* fruit set between five different pollination treatments in 2008/09. Shown are 95% confidence intervals and respective P values.

Treatments compared	Difference	Lower CI	Upper CI	P
Caged vs. Bagged	0.00	-24.81	24.81	1.000
Crossed vs. Bagged	36.00	11.19	60.81	0.003
Natural vs. Bagged	40.00	15.19	64.81	0.001
Selfed vs. Bagged	32.00	7.19	56.81	0.009
Crossed vs. Caged	36.00	11.19	60.81	0.003
Natural vs. Caged	40.00	15.19	64.81	0.001
Selfed vs. Caged	32.00	7.19	56.81	0.009
Natural vs. Crossed	4.00	-20.81	28.81	0.987
Selfed vs. Crossed	-4.00	-28.81	20.81	0.987
Selfed vs. Natural	-8.00	-32.81	16.81	0.857

Agelanthus djurensis

Flower opening

In 2008/09 240 *A. djurensis* flower buds were selected for the flower opening experiment of which the fate of 235 buds was successfully recorded.

Once again, analysis revealed a highly significant treatment effect on flower opening, whilst variation between plants was not significant (Table 2.22).

Table 2.22: Results from the GLM ANOVA model testing *A. djurensis* flower opening under bagged, caged and natural pollination treatments across eight plants in 2008/09. A binomial error distribution was used with a chi-squared test for significance.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			23	248.66	
Plant	7	2.79	16	245.87	0.904
Treatment	2	234.44	14	11.44	P < 0.001

On average, only 5.1% of bagged flowers of known fate opened, indicating that the flowers of *A. djurensis* do not self-open. In contrast, 94.9% of flowers inside cages and 97.4% of natural flowers were opened (Fig. 2.30). Results of the Tukey's HSD test confirm there is no significant difference in the proportion of flowers opened under the caged and natural conditions (Table 2.23), suggesting that insects are equally as effective as sunbirds at opening the flowers of *A. djurensis*.

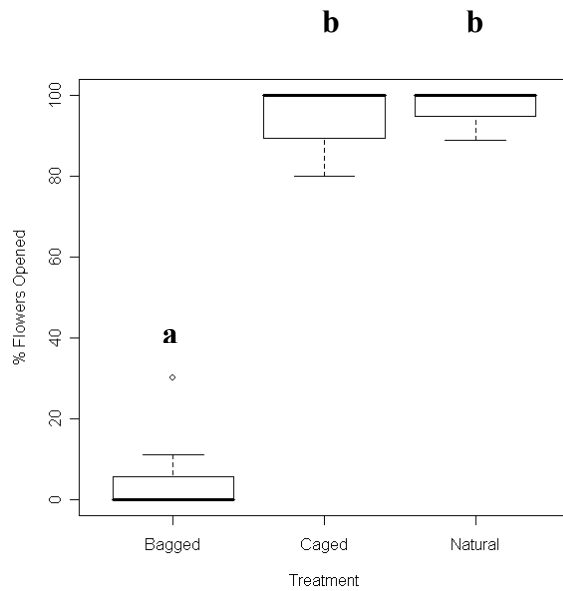


Figure 2.30: Percentage of *A. djurensis* flowers opened under three pollination treatments in 2007/08. Circle represents an outlier. Each box shows median percentage of flowers opened and the 95th, 75th, 25th and 5th percentiles). Number of flowers opened = 154 of 235 (66%). Results from Tukey's HSD test are also indicated as letters above boxes.

Table 2.23: Results from Tukey's HSD multiple comparison test comparing *A. djurensis* flower opening between pollination treatments. Shown are 95% confidence intervals and respective P values.

Year	Treatments compared	Difference	Lower CI	Upper CI	P
2008/09	Caged vs. Bagged	89.75	79.86	99.64	P < 0.001
	Natural vs. Bagged	92.25	82.36	102.14	P < 0.001
	Natural vs. Caged	2.50	-7.39	12.39	0.789

Fruit set

Of the eight *A. djurensis* plants used in the fruit set experiment, one plant died after the flower opening experiments. Results presented are from the remaining seven plants comprising a total of 350 flower buds. In total, 121 of these 350 (35%) *A. djurensis* flowers produced fruit across the five pollination treatments.

Analysis revealed that treatment had a highly significant effect on fruit set, whilst variation in fruit set between study plants was not significant (Table 2.24).

Table 2.24: Results from the GLM ANOVA model comparing *A. djurensis* fruit set versus failure under bagged, caged, natural, selfed and hand-crossed pollination treatments across seven plants in 2008/09. A binomial error distribution was used with a chi-squared test for significance.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			34	121.824	
Plant	6	10.130	28	111.694	0.119
Treatment	4	77.896	24	33.798	P < 0.001

Fruits were almost never produced inside a bag (2.9%) indicating *A. djurensis* is not autonomous (Fig 2.31). Insects do not play a significant role in fruit production as there was no significant difference between the bagged and caged pollination treatments (Table 2.25). Fruit set under natural conditions (54.3%) was significantly higher than under the bagged or caged conditions (Fig 2.31), indicating that sunbirds play the most important role in fruit production of this species. Hand-selfed flowers produced fruit 50% of the time revealing *A. djurensis* is highly self-compatible. A Tukey's HSD test confirmed no significant difference in fruit set under the hand-crossed (47.1%), natural and hand-selfed pollination treatments indicating that in 2008/09, *A. djurensis* was not pollen limited (PLI=0) (Table 2.25).

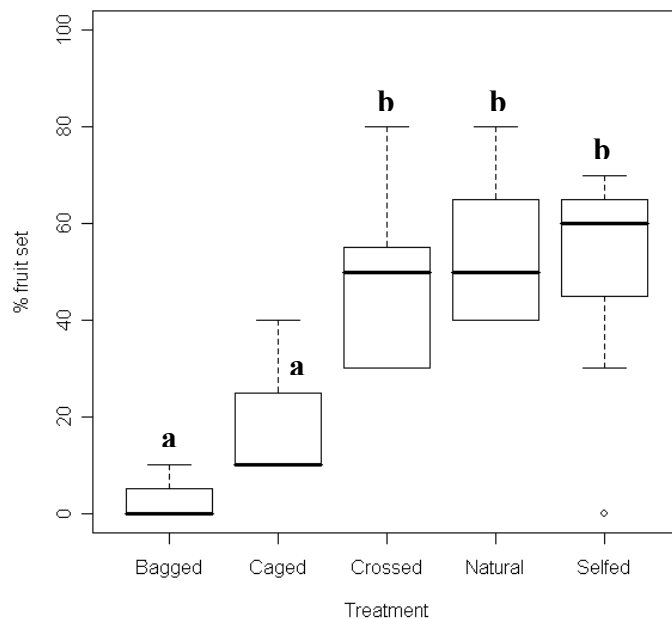


Figure 2.31: Percentage of *A. djurensis* flowers that set fruit under five pollination treatments in 2008/09 across seven study plants (n=350). Each box shows median percentage of flowers that produced fruit and the 95th, 75th, 25th and 5th percentiles. Circle represents an outlier. Results from Tukey's HSD test are also indicated as letters above boxes.

Table 2.25: Results from Tukey's HSD multiple comparison test comparing *A. djurensis* fruit set between pollination treatments in 2008/09. Shown are 95% confidence intervals and respective P values.

Treatments compared	Difference	Lower CI	Upper CI	P
Bagged vs. Selfed	-47.14	-73.01	-21.23	P < 0.001
Caged vs. Selfed	-31.43	-57.29	-5.56	0.012
Crossed vs. Selfed	-2.86	-28.72	23.01	0.997
Natural vs. Selfed	4.29	-21.58	30.15	0.988
Caged vs. Bagged	15.71	-10.15	41.58	0.402
Crossed vs. Bagged	44.29	18.42	70.15	P < 0.001
Natural vs. Bagged	51.43	25.56	77.29	P < 0.001
Crossed vs. Caged	28.57	2.71	54.44	0.025
Natural vs. Caged	35.71	9.85	61.58	0.004
Natural vs. Crossed	7.14	-18.72	33.01	0.924

2.8 Discussion

2.8.1 Flower development

The flower petals of the three Ngel Nyaki mistletoe species remained attached to the plant for on average 11-21 days. Ashman & Schoen (1996) reviewed floral longevity in 280 flowering plants and showed that over 90% of flowers lasted less than 10 days, with the majority lasting no longer than 4 days after opening. Studies of other Loranthaceous mistletoes have reported floral longevities ranging from one day to 12 weeks (Docters Van Leeuwen 1954, Davidar 1984, Rivera et al. 1996, Vaknin et al. 1996, Ladley et al. 1997, Aizen 2003, Azpeitia and Lara 2006). The flowers of the *Agelanthus* mistletoes changed colour as the flower aged, presumably as a cue to pollinators signaling diminishing nectar rewards. It would seem that the abscission of old flower petals would serve the same purpose. However there are several proposed advantages of retaining old, post-pollinated flowers on plants.

Vatkin et al. (1996) propose that flower longevity of *Loranthus acaciae* increases the overall attractiveness of a plant to pollinators by maintaining a large floral display with old flowers being retained as "flags". This strategy potentially provides the plant with an advantage when competing for pollinators, and once at the plant, differences in floral colour may direct pollinators to flowers presenting larger nectar rewards (Gori 1983). Studies on the Australian Loranthaceous mistletoes *Amyema* found a positive relationship between the number of open flowers a species presents on an inflorescence and branch, and the number of birds visiting those plants. Birds were

also observed shifting to co-blooming species bearing a larger number of open flowers per branch (Bernhardt et al. 1980, Bernhardt 1983). A study of floral colour changes of an endemic New Zealand tree *Fuchsia excorticata* confirmed that change in colour of flowers from green to red was age-dependent, but found no effect on pollen deposition onto green-phase flowers after removing the red flowers (Delph and Lively 1989). Delph & Lively (1989) instead argued that the old red flowers are retained long enough for pollen tubes to grow the length of the long style before complete abscission of the petals and style. In the three Ngel Nyaki mistletoe species, styles are generally shed with the petals, however retention of the style after the petals were shed was sometimes observed.

In higher elevations, a longer life-span may be selected for due to cooler, wetter and more unpredictable weather conditions and an associated scarcity of biotic pollinators. Remaining open for a longer period under such conditions may increase the number of pollinator visits a flower receives. Individual flowers in subalpine and montane forests have been reported as being retained significantly longer than those in adjacent lowland forests, with tropical forests retaining flowers for one day on average compared with 4-14 days in montane and subalpine habitat (Primack 1985, Stratton 1989). Flowers of *Tristerix corymbosus* are typically open for one week in the Southern Andes during the summer months, but lasted 2-3 times longer during the winter months (Aizen 2003). Floral longevity has however been recorded for mistletoe species in lowland habitat, such as the flowers of *L. acaciae* which turn red when within 1–2 days of being pollinated and remain on the plant for 6–12 weeks (Vaknin et al. 1996).

Kirkup (1993) identified a significant relationship between the restriction of access of the pollinator to nectar and the volume of nectar produced in the closed flowers of African Loranthaceae. Restriction of access was expressed as a distance measurement of the position of the initial probing from the base of the flower. This relationship was found to be positive; i.e. pre-anthesis nectar production increased as restriction of access to the nectar increased. This trend does not explain the high pre-anthesis nectar production in *G. braunii*, but restriction of access may be underestimated in this genus due to the two-stage opening process.

Pre-anthesis signals are present in all three species upon ripening, with darkening of the mature flower bud heads in *G. braunii*, basal swelling and the formation of fenestrae in *A. brunneus*, and darkening of the bud tip and fenestrae formation in *A. djurensis*. Post-anthesis colour signaling appeared absent in *G. braunii* but is highly developed in the *Agelanthus* genera. Brightly

coloured throat patterns exposed during opening fade as the flower ages, and the whole flower flushes pink. These colour changes presumably signal to potential pollinators that the flowers no longer contain nectar. Correlation between flower colour patterns and nectar production has been identified in other studies of African mistletoes (Gill and Wolf 1979, Kirkup 1993). Further experiments on stigma receptivity and pollen tube development need to be conducted on the Ngel Nyaki mistletoes to increase understanding of the association between floral development, nectar production and pollination.

In terms of structural complexity and colour patterning, *Globimetula* appears to be less specialised than *Agelanthus*, as has been noted by previous studies of these genera (Kirkup 1993, 1998). Within the explosive African Lorantheaceae with fenestrate, *Agelanthus* is considered to represent an evolutionary end point (Kirkup 1998).

2.8.2 Role of pollinators in flower opening

Flower-opening is the necessary first step in pollination of the Ngel Nyaki mistletoes as fruits were almost never produced within closed flowers. Some interesting discoveries were made within this part of the study. Firstly, flowers of *Agelanthus* required probing by pollinators to open, whereas flowers of *Globimetula braunii* were able to self-open 66% of the time on average. Pollinators did however increase both the proportion of buds opened and the rate (speed) of opening in *G. braunii*. It could not be determined whether all four sunbird species were opening the flowers. Only the largest two sunbird species from the genus *Cyanomitra*, *C. obscura* and *C. verticalis*, with the longest heaviest bills (Cheke and Mann 2001) were actually observed flower opening. *C. obscura* was also frequently observed with a significant dusting of pollen on its crown feathers. Previous studies of Lorantheaceae have shown flower-opening to be related to pollinator size, a relationship which can be explained by an increased selection pressure on larger birds associated with their higher energetic requirements (Gill and Wolf 1975). If only two of the four sunbird pollinators observed are actually opening the flowers, then the guild of pollinators transferring pollen may be reduced. This is because the majority of pollen is released explosively from the anthers onto pollinators during opening. In *G. braunii* especially, the tight curling of the stamen filaments after opening means that even if the anthers did retain some pollen after opening, they would unlikely be in a position where they would contact subsequent flower visitors. This is not to say that the event of opening itself is sufficient for successful pollination,

and several subsequent visits may be required to effect pollen tube growth. This has been shown in New Zealand for *Peraxilla* flowers, which often fail to receive any pollen grains on the stigma during opening, and rely on subsequent accumulation of pollen over 6–7 days after flower opening for successful pollination (Robertson et al. 1999, Robertson et al. 2005). Further work with high-speed camera gear would be valuable to compare flower opening between sunbird species.

Secondly, insects were found to be just as effective in opening the flowers of *Agelanthus* as sunbirds, and a small Vespinae wasp species was observed opening the flowers of *A. djurensis*. Flower opening of explosive bird-pollinated flowers by invertebrates has to my knowledge only been recorded once elsewhere; the small native solitary bee *Hylaeus agilis* and an undescribed species of *Leioproctus* have been observed prising open the flowers of *Peraxilla* mistletoes in New Zealand (Kelly et al. 1996, Robertson et al. 2005). The Vespinae observed opening the flowers of *A. djurensis* were clearly opening the flowers just enough to harvest pollen from them and therefore are unlikely to be in direct competition with the sunbirds for nectar. However, by removing the majority of pollen from the flowers prior to anthesis and only effecting limited fruit set the wasps may be limiting the reproductive potential of *A. djurensis*. Pollen consumption by flower visitors has been shown to negatively affect reproductive performance in a wide range of other angiosperm families (Hargreaves et al. 2009). It is possible that the wire cages deterred some insect visitors from visiting the flowers within. I observed the Vespinae moving freely in and out of the cages and their behaviour appeared unimpeded by the presence of the cage. Previous studies have tested for the effects of caging on insect visitation to mistletoe flowers and found no significant difference in visitation rates to flowers within and outside of cages (Robertson et al. 2005).

The discovery that *Apis mellifera* were robbing nectar from the flowers of *A. brunneus* requires further investigation. Whether the bees were primary or secondary nectar robbers remains unknown. However, as *A. mellifera* lacks biting mouthparts (Proctor et al. 1996) it seems probable they were acting as secondary nectar robbers, as has been recorded on other flowering plants (Maloof and Inouye 2000, Sampson et al. 2004, Goulson et al. 2007).

It is possible that the presence of the pollination treatments also altered the behaviour of the sunbirds when foraging. I spent several hours observing mistletoe plants following the application of the treatments and this did not appear to be the case. The sunbirds seemed

undeterred by the presence of the pollination treatments on the plants and moved freely amongst the treatments whilst visiting flowers (see Fig 2.18).

2.8.3 Breeding systems and the role of pollinators in reproductive output

The flowers of *G. braunii*, *A. brunneus* and *A. djurensis* almost never set fruit when pollinators were excluded, revealing that these species are not autonomous and require biotic pollinators to achieve their reproductive capacity. However, hand-selfed flowers resulted in fruit production either equal or greater (*G. braunii*) than that achieved by natural pollination revealing that the Ngel Nyaki mistletoes are clearly self-compatible. Self-compatibility appears to be common in the Loranthaceae (Docters Van Leeuwen 1954, Bernhardt 1983, Rivera et al. 1996, Vaknin et al. 1996, Ladley et al. 1997, Aizen 2005, Azpeitia and Lara 2006).

Outbreeding is considered to be the norm within the Loranthaceae (Barlow 1983). The fact that the Ngel Nyaki mistletoes are highly self-compatible yet lack autonomy indicates the presence of either a structural (herkogamy) or temporal (dichogamy) mechanism preventing autonomous selfing. Morphological assessment of the African Loranthaceae indicates herkogamy and dichogamy are features of all explosive genera (Kirkup 1993). The anthers usually surround the style in a tight column positioned immediately below the stigma. The stigmatic papillae also appear crowded together at anthesis, only later separating enough for pollen to lodge between the papillae bases (Kirkup 1993). Herkogamy has also been cited as the main mechanism preventing spontaneous selfing in Loranthaceae elsewhere (Rivera et al. 1996, Tadey and Aizen 2001, Aizen 2005). Other self-compatible Loranthaceae have been found to exhibit strong protandry, such as the Australian genus *Amyema* (Bernhardt 1983) and *Psittacanthus calyculatus* in Central Mexico (Azpeitia and Lara 2006). Azpeitia and Lara (2006) found the stigmas of *Psittacanthus calyculatus* to be receptive two days after flower opening. Hand-pollinations of flowers in the present study were performed both on opening and two days after opening in an attempt to ensure stigma receptivity when testing for self-compatibility. Repeating the hand-pollinations two days after opening in the current study may have obscured any dichogamous outbreeding mechanisms present. Stigma receptivity in the Ngel Nyaki species should be investigated in future work. Testing for inbreeding effects within the Ngel Nyaki mistletoes by comparing seed germination and growth of seedlings between outcrossed and selfed fruits would also provide further insight

into the adaptive nature of maintaining a predominantly outbreeding reproductive system within these plants.

Although insects were effective at opening the flowers of *Agelanthus*, it appears they play an insignificant role in fruit set and all three species are almost entirely reliant on 3 – 4 sunbird species for fruit production. Interestingly, only one of the three Ngel Nyaki mistletoes, *G. braunii*, was found to be pollen limited (PLI=0.504). The average PLI for 224 species of angiosperms reviewed by Larson and Barrett (2000) was 0.404 (± 0.022). The discovery that *G. braunii* flowers are able to self-open might have suggested that this species would be less susceptible to pollinator limitation than the *Agelanthus* spp. but this was clearly not the case. The Patagonian Loranth *Tristerix corymbosus* becomes pollen limited during winter flowering due to reduced visitation by hummingbirds (Aizen 2003). In New Zealand Loranaceae, pollen-limitation has been attributed to a decline in pollinating bird species as a result of predation by introduced mammals (Robertson et al. 1999). For *G. braunii*, it may be that during peak flowering in December, the sunbirds were foraging on other more energetically rewarding resources ‘distracting’ them from *G. braunii* (Gross and Werner 1983, Campbell 1985, Gross 1996, Murphy and Kelly 2001). A large endemic tree *Anthonothea noldeii* (Fabaceae) was in full flower at this time and large numbers of sunbirds were observed to be foraging on *A. noldeii* around the forest edge (pers. obs). However, observed sunbird visitation rates at *G. braunii* were similar to those of the *Agelanthus* spp., with 0.107 visits per flower per hour equating to about 14 seconds of visitation time per flower over its lifetime. These visitation rates are also similar to visitation rates recorded for the New Zealand Loranaceae *P. colensoi*, which was found not to be pollen limited during that study (Robertson et al. 1999). It seems unlikely that the low fruit set under natural conditions is a product of low quality selfed pollen reaching the stigmas of *G. braunii* due to the fact that there was no difference in fruit production between hand-selfed and hand-crossed flowers. However, it is possible that the less specialised actinomorphic flowers of *Globimetula* result in less precise pollen placement, and consequently a less efficient pollination mechanism than that of the *Agelanthus* spp. The brief nectar schedule of *G. braunii* flowers after opening relative to the *Agelanthus* spp. may also limit ongoing visitation to already open flowers, thereby resulting in lower female reproductive success of *G. braunii* relative to the *Agelanthus* spp. One final possibility is that *G. braunii* serves as an early indicator of ecological disruption, which may ultimately have consequences on all three mistletoe species over time.

This study was limited to plants located along the forest edge and consequently reproductive output may have been biased by edge effects (Murcia 1995). A recent literature review of edge effects on plant reproduction classified species as showing a significant negative effect, no effect, or a significant positive effect and found 69% of species showed some effect, the majority of which were negative (Burgess et al. 2006). However, studies of the New Zealand Loranthaceae *Peraxilla tetrapetala* have shown higher fruit set in plants growing along forest edges attributed to higher visitation rates by birds and bees (Kelly et al. 2000, Burgess et al. 2006). Although African Loranthaceae often occur most abundantly along forest edges (Polhill 1989), future studies could aim to access plants high within the canopy of the forest interior to compare the pollination biology of these plants to those included in the present study. The size and density of the three populations should also be assessed to determine whether differences in population structure can further explain the observed pollen limitation in *G. braunii* comparative to the other Ngel Nyaki mistletoe species studied.

2.8.4 Conclusions

The floral ecology of the Ngel Nyaki mistletoes appears to be mutualistically congruent with their principal pollinators, the sunbirds. Flower development, particularly within *Agelanthus*, looks to be highly evolved with a concealed nectar reward inside the mature bud, a complex explosive flower opening mechanism, highly refined morphological and colour signaling both pre- and post-anthesis and relatively long-lived flowers. The flower buds of *G. braunii* are able to self-open, but the proportion and speed of flower opening is significantly increased when sunbirds have access to the flowers. In contrast, the flowers buds of the *Agelanthus* species require probing by pollinators to open, and although conforming to an ornithophilous pollination syndrome, a small species of social wasp in the subfamily Vespinae is equally as effective in opening the flowers. Hand-pollinations of all three species indicated a high level of self-compatibility and in one species, *G. braunii*, pollen limitation was evident. There was no significant evidence for autonomous selfing within any of the Ngel Nyaki species, and although insects were effective in opening the flower buds of the *Agelanthus spp.*, fruit production in all three mistletoe species is almost entirely reliant on 3 – 4 species of closely related sunbirds. Although the importance of the mistletoes as a resource for the sunbirds remains unknown, this work reveals that the sunbirds are of vital importance to the pollination of these plants. The specificity of this pollination mutualism, combined with the plants high dependence on this process

due to a lack of vegetative reproduction and autonomous self-pollination makes it highly susceptible to failure should the mistletoes' sunbird mutualists decline (Bond 1994).

Chapter 3: Dispersal Ecology

3.1 Introduction

The dispersal system of any plant primarily functions to facilitate colonisation of new areas, allow seeds and seedlings to escape from disproportionate mortality beneath the parent plant, reduce sibling competition or enable seeds to reach microhabitats suitable for establishment and growth (Howe and Smallwood 1982). Limitation of recruitment can occur if the dispersal system fails to meet the requirements of the plant in terms of both the quality and quantity of seeds dispersed to potential recruitment sites, resulting in what is commonly termed ‘dispersal limitation’ (Schupp 1993). The quality of seed dispersal, or the probability that a dispersed seed will result in a new reproductive adult, depends on the quality of treatment and deposition site of the seeds. Seed treatment by frugivores can enhance seed germination by three mechanisms: (1) passing through the frugivores gut may have an abrasive or ‘scarification effect’ on the seed coat thereby increasing permeability of the coat to water and gases; (2) separation of the seeds from pulp may decrease the likelihood of microbial or fungal attack and/or remove chemicals which may otherwise inhibit germination, known as the ‘deinhibition effect’; and (3) when defecated, there may be a ‘fertilisation effect’ as the seed is surrounded with nutrient- and microbial-rich faecal material (Traveset and Verdu 2002).

Traveset & Verdu (2002) conducted a meta-analysis of the effect of gut treatment on final percentage seed germination using the results of 83 studies covering 213 plant species. The effect of gut treatment on the rate of seed germination was also tested for 30 experiments across 16 plant species. Effects were calculated for each experiment as an odds ratio estimate i.e. the probability of germination after gut treatment relative to the probability of germination in the control group. When individual effect sizes were combined, the overall effect of gut treatment on percentage seed germination was significantly positive. However, variance between individual effect sizes was greater than expected indicating that other variables were influencing the result. A variety of predictor variables were tested, all of which were found to be significant; these were the frugivore taxon, fruit type and size, plant type (shrubs versus trees), biome, habitat and experimental conditions. The overall speed at which seeds germinate was also found to be significantly affected by gut treatment, however due to the small number of replicated

experiments testing germination rate, the effects of different variables were not tested. The majority of studies included in their meta-analysis compared germination of bird-defecated versus hand-cleaned seeds and did not include germination in intact fruits. Subsequent studies have stressed that in order to evaluate the effects of gut passage on germination more completely, experiments should also address germination in intact fruits (Kelly et al. 2004, Samuels and Levey 2005, Robertson et al. 2006). Robertson et al (2006) reviewed the relative effects of scarification and deinhibition effects of 51 plant species and found that deinhibition effects are in fact often greater than the more widely measured scarification effects. How and where seeds are deposited also affects the quality of seed dispersal and is largely dependent on patterns in movement of the dispersers and whether seeds are regurgitated or defecated (Davidar 1983a, Schupp 1993, Lambert 2002, Wang and Smith 2002, Roxburgh 2007).

Quantity of seed dispersal is affected by the number of visits dispersers make to a plant and the number of seeds removed per visit (Schupp 1993). Consequently, one component of dispersal limitation is 'disperser limitation'. The clearest evidence for disperser limitation is finding that a high proportion of a fruit crop remains unconsumed by potential seed dispersers (Corlett 2007). The proportion of the total fruit crop that is removed from an individual plant can be defined as 'dispersal efficiency' (Willson and Whelan 1993). Fruit removal efficiency varies extensively both within and between plant species and can be affected by fruit traits (Izhaki 2002), disperser activity (Herrera 1984, Chapman and Chapman 1996), habitat fragmentation (Cordeiro and Howe 2001, Cordeiro and Howe 2003, Galetti et al. 2003, Farwig et al. 2006, Rodriguez-Cabal et al. 2007, Kirika et al. 2008), season (McCarty et al. 2002), competition for dispersers (Manasse and Howe 1983, Wheelwright 1985a, Carlo 2005), insect damage (Jordano 1987a), the presence of secondary metabolites (Cipollini and Levey 1997) and fruit crop size (Davidar and Morton 1986, Jordano 1987a, Murray 1987, Willson and Whelan 1993, Saracco et al. 2005); all of which are not mutually exclusive. Furthermore, fruit removal may also vary between lowland and montane habitat (Herrera 1995).

Whilst most dispersal mutualisms tend predominantly towards generalisation, plants exhibiting 'directed dispersal' can be exceptions to this rule (Howe and Smallwood 1982, Wheelwright and Orians 1982, Herrera 2002). In these cases, dispersers deposit seeds in non-random sites suitable for establishment and growth, thereby increasing plant fitness through high quality dispersal (Wenny 2001). This increase in plant fitness may provide the selective pressure for more

specialised plant-disperser relationships to coevolve, as is often observed between mistletoes and their avian dispersers (Reid 1989, 1991, Sargent 1995, Lopez de Buen and Ornelas 1999, Green et al. 2008). A specialised dispersal system, or relying on just one or a few species may come at a cost however, should these dispersal mutualists become limited (Bond 1994, Corlett 2007).

3.2 The Loranthaceae fruit

The Loranthaceae fruit is technically referred to as a ‘pseudoberry’ due to a complete lack of true ovules, and therefore, true seeds. The term seed can, however, be applied to the functional unit (Kujit 1969). Loranthaceae are unique amongst the mistletoes in that several embryo sacs develop concurrently (Bhatnagar and Johri 1983). Only one embryo sac generally reaches full development, although several may contribute to the compound endosperm (Calder 1983). Surrounding the bright green embryo is an endocarpic region consisting of a sticky viscin layer and an outer ‘fleshy layer’ composed of nutritious materials. The viscin layer varies between species in both quantity and position around the seed, and serves to anchor the seed to a host branch after being voided by birds either by regurgitation or defecation. The exocarpic skin of the Loranthaceae fruit is usually smooth and leathery, also varying in colour and thickness between species (Kujit 1969). Fruit lengths within the family have been recorded between 3 – 15 mm and seeds 2- 12 mm (Ali 1931, Docters Van Leeuwen 1954, Kujit 1969, Bhatnagar and Johri 1983, Godschalk 1983a, Hoffmann et al. 1986, Reid 1986, Dowsett-Lemaire 1988, Polhill 1989, Ladley 1994, Martinez del Rio et al. 1995, Ladley and Kelly 1996, Lopez de Buen and Ornelas 1999, Amico and Aizen 2000). The fruits of one primitive genus of root-parasites, *Nuytsia*, are a known exception within the family being dry, winged and dispersed by wind (Calder 1983, Lamont 1985).

Germination appears to be entirely dependent on removal of the seed from the exocarpic layer of the fruit, which under natural conditions occurs either during gut passage or regurgitation (Calder 1983, Ladley and Kelly 1996). Once this has occurred, the seeds usually begin to germinate immediately. During germination, the hypocotyl shoot with the radicle at the tip pushes out of the endosperm and grows rapidly towards the substrate. The ability of the radicle to find the host is thought to be a combination of negative phototropism and negative geotropism. On contact with

the substrate a small attachment disc develops. After a long delay, a small proportion achieve penetration through the surface and establishment of xylem-xylem contact (Kujit 1969).

3.3 Dispersal mutualisms in Loranthaceae

3.3.1 Worldwide

Interdependence between Loranthaceous mistletoes and their local set of largely avian dispersers is often highly specialised, with this close affinity cited as contributing to wide diversification within the family (Reid 1990, Restrepo et al. 2002, Green et al. 2008).

Different assemblages of birds dispersing the seeds of Loranthaceae worldwide have been recognised as handling the fruits and seeds in three ways; regurgitation, defecation and pecking (Davidar 1983a, Godschalk 1985). Each of these methods varies in both the quality and quantity of consequent seed dispersal. During regurgitation, the sticky seed generally adheres to the birds' bill forcing the bird to remove it by rubbing it off onto a branch. In contrast, defecated seeds are either defecated haphazardly beneath the bird, or the birds are forced to vigorously rub their bodies against a branch in an attempt to remove the viscous mass of seeds from their cloaca (Davidar 1983a).

Roxburgh (2007) investigated the quality of seed dispersal of the Loranthaceous mistletoe *Phragmanthera dschallensis* and found that regurgitated seeds had almost twice the final percentage germination as defecated seeds. Several mechanisms for higher percentage germination of regurgitated seeds were identified. Defecated seeds were usually clumped and had less direct contact with the host branch compared with regurgitated seeds, the host branch was less likely to be that of another mistletoe if seeds were regurgitated, and host branch size was significantly smaller for regurgitated seeds. Host branch size has previously been shown to positively affect mistletoe establishment and is attributed to larger branches having thicker bark that is more difficult for the germinating mistletoe to penetrate (Sargent 1995, Norton et al. 1997, Norton and Ladley 1998). Other studies on New Zealand Loranthaceae have reported no significant effect of branch size (Ladley and Kelly 1996). Davidar (1983a) compared the distribution of seedling, juvenile and adult plants of two neotropical mistletoes *Phoradendron quadrangulare* (Viscaceae), the seeds of which are dispersed by defecation and *Oryctanthus*

alveolatus (Loranthaceae) which is dispersed by regurgitation. Comparisons between species showed that significantly more seeds of the defecated species *P. quadrangulare* were clumped. No correlation was found between the initial number of seeds in a clump and the percentage which germinated for either species. However, for both species, significantly more seeds were distributed in clumps than juveniles or adult plants, the latter of which were mostly distributed singly. Davidar (1983a) purported that high post dispersal density dependent mortality affected the two species disproportionately, with mortality being higher in the defecated species *P. quadrangulare*, as inferred from the preponderance of singly distributed adults of both species. Kelly et al (2007) found that seeds of the New Zealand mistletoe *Peraxilla tetrapetala* which were deposited at higher densities were more likely to achieve adhesion to the host branch. However, no significant density effects on survival of the seeds were found, providing strong evidence for seed limitation. By the end of the six year study, most surviving seedlings were distributed singly, irrespective of whether they began in high or low density clumps. This result is similar to the findings of Davidar (1983a) for juvenile and adult mistletoe distribution. However Kelly et al (2007) did not follow deposited seeds through to adulthood due to the slow growth rate of *P. tetrapetala* and the fact that high mortality rates resulted in single distribution for the majority of surviving seedlings after six years.

The mode of seed deposition and specificity of the relationship between mistletoes and their avian dispersers may be related to fruit structure (Davidar 1983a). Davidar (1983a) suggested that in the neotropics, viscaceae fruits with ‘hard’ exocarps are generally ingested and defecated by specialised frugivores from a single taxon, the euphonias (Fringillidae). In contrast, fruits with ‘soft’ exocarps are usually associated with a more generalised guild of frugivores which regurgitate the seeds. Although precise definitions of the terms ‘hard’ and ‘soft’ are not provided, Davidar (1983b) describes the fruits of Indian Loranthaceae defecated by the flowerpeckers as having a hard-rinded and cutinized exocarp. Davidar (1983a) proposes that in fruits with hard exocarps, the inner pulpy mass remains firmly adhered to the seed upon ingestion and gut passage functions to separate the pulp from the seed, which is subsequently excreted. In contrast, in fruits with soft exocarps, the pulp adheres to the digestible exocarp and is easily separated from the seed. The seed can therefore be regurgitated quickly, or squeezed out from the fruit and wiped off onto a branch. The specialised relationship between the neotropical Loranth *Psittacanthus robustus* and its avian dispersers, three species of tanager (Thraupidae), does not support this pattern however (Monteiro et al. 1992). The tanagers belong to a single taxon yet

they regurgitate the seeds of *P. robustus*. Furthermore, only one tanager species, *Tersina viridis* is considered as effective in dispersing the seeds. This is in contrast to the pattern proposed by Davidar (1983a) whereby regurgitation is associated with a generalised guild of frugivores. Reciprocally, a wide range of avian dispersers have been recorded as defecating the seeds of New Zealand Loranthaceae, the fruits of which do not have a hard-rinded cutinized exocarp (O'Donnell and Dilks 1994, Ladley and Kelly 1996).

In the neotropics, Loranthaceae seeds are generally regurgitated by their avian dispersers, including the flycatchers (Tyrannidae) (Davidar 1983a, Lopez de Buen and Ornelas 1999), tanagers (Thraupidae) (Snow and Snow 1971, Monteiro et al. 1992), cotingas (Contingidae) (Parker 1981) and thrushes (Turdidae) (Hoffmann et al. 1986). The endemic Central Chilean mistletoe *Tristerix aphyllus* is an exception to this pattern, as it is dispersed exclusively by the mockingbird *Mimus thenca* (Mimidae), which either regurgitates or defecates the seeds onto two cacti host species *Echinopsis chilensis* and *Eulychinia acida* (Martinez del Rio et al. 1996, Soto-Gamboa and Bozinovic 2002, Medel et al. 2004). Soto-Gamboa & Bozinovic (2002) found no significant difference in the percentage of *T. aphyllus* seeds which germinated when regurgitated compared with those defecated. However, radicle growth rate of defecated seeds was found to be significantly higher during the nine day experiment. Soto-Gamboa and Bozinovic (2002) claim to demonstrate the importance of different modes of fruit processing for infection efficiency by the mistletoe, although they did not specify the mechanism by which seeds with a longer radicle achieve greater establishment success. It may be that a longer radicle provides an advantage to seeds which are deposited onto spines by enabling them to grow down and establish contact with the epidermis of the cactus. Previous studies have shown the radicle of *T. aphyllus* grows to a length of approximately 4 cm over a period of up to 8 weeks, or until it encounters the host epidermis (Martinez del Rio et al. 1995, Martinez del Rio et al. 1996). Given that the duration of this experiment was nine days, it is possible that the radicle of regurgitated seeds is able to grow equally as long, over a longer time period, and will still establish contact with the host epidermis, despite the slower initial growth rate found in this study.

Another unique dispersal mutualism has been recorded in a temperate South American forest; that is the only case of Loranthaceae dispersal by a marsupial (Amico and Aizen 2000). The arboreal marsupial *Dromiciops australis*, endemic to the Lake District of Southern Argentina, is the only recorded disperser of the seeds of *Tristerix corymbosus* which it defecates undamaged.

Passage of seeds through the marsupial's gut is essential for germination and developing a holdfast for attachment to the host plant. A recent study assessing the effects of fragmentation on this mistletoe-disperser mutualism found fruit removal rates, seed dispersal and seedling recruitment were significantly lower in fragmented compared with continuous forest (Rodriguez-Cabal et al. 2007). These effects on *T. corymbosus* dispersal were closely related to negative impacts of forest fragmentation on the abundance of its sole disperser, *D. australis*.

Throughout Indo-Malaya, mistletoe dispersal is performed almost exclusively by the flowerpeckers (Dicaeidae) (Ali 1931, Ali 1932, Docters Van Leeuwen 1954, Davidar 1983b, Devkota and Glatzel 2005). The flowerpeckers characteristically handle the mistletoe fruits by squeezing the pulp-covered seed from its exocarp and swallowing it, sometimes rubbing the discarded exocarp off onto a branch. Ali (1931) describes how after 4 or 5 seeds have been consumed, the bird appears to sit and wait for 3 - 4 minutes until the seeds pass through their digestive system. Docters van Leeuwen (1954) recorded a gut passage time of approximately 12 minutes for the flowerpecker *Dicaeum sanguinolentum*. The digestive system of the flowerpecker is specially adapted to the fast passage of mistletoe seeds, with the seeds completely by-passing the gizzard (Richardson and Wooller 1988). Upon defecation of the seeds, the flowerpecker vigorously rubs the seeds off against a branch, although some seeds may be defecated in flight, which may be important for long-distance dispersal (Ali 1931, Docters Van Leeuwen 1954).

Flowerpeckers, together with honeyeaters (Meliphagidae) are also the principal dispersers of mistletoe fruits in Australia (Liddy 1983, Reid 1986, 1987, 1989, 1990, 1991, Yan 1993). The seeds of several Loranthaceae from the genera *Muellerina*, *Lysiana* and *Amyema* in South Australia are dispersed primarily by the mistletoe bird *Dicaeum hirundinaceum* and to a lesser extent, the spiny-cheeked honeyeater *Acanthagenys rufogularis*. The Australian flowerpeckers handle the mistletoe fruits in a similar way to the Indo-Malayan Dicaeidae, however the honeyeaters usually defecate the seeds clear of their perch and are therefore considered to be less effective seed dispersers (Reid 1989).

In New Zealand, five extant Loranthaceae are each dispersed predominantly by 2 - 5 species of bird, the most important of which are members of the Meliphagidae and Zosteropidae (Ladley and Kelly 1996). All New Zealand Loranthaceae dispersers swallow the fruits whole and subsequently defecate the seeds, with no instances of regurgitation recorded. Seed dispersal was found to be limited at one New Zealand site by assessing fruit ripening and removal rates (Ladley

and Kelly 1996). This dispersal limitation is likely to be attributed to a recent decline in the mistletoes native bird dispersers due to the introduction of introduced predatory mammals (Ladley and Kelly 1996, Murphy and Kelly 2001, Kelly et al. 2004). Studies of New Zealand mistletoes also showed removal of the exocarp is required for germination, but gut passage does not improve germination, as shown by comparisons between intact, hand-cleaned and bird-cleaned fruits (Ladley and Kelly 1996). Studies to date indicate that mistletoes do not germinate within the intact fruit, however on excision of the seed from the exocarp, germination is triggered almost immediately (Lamont 1983). Study of the mature fruits of Australian Loranthaceae *Ameyma preissii* suggest that gas exchange through the exocarp is the principal factor preventing germination within the fruit (Lamont and Perry 1977, Lamont 1983). Carbon dioxide concentration within the fruit is high (27%) and oxygen influx is prevented, both of which appear to inhibit photosynthesis and respiration of the seed until the exocarp is removed and the gases can freely exchange.

Studies of Loranthaceae in the Middle East are rare; however recent work investigated the dispersal system of *Plicosepalus acaciae* in the Syrian–African Rift (Arava) valley, Israel (Green et al. 2008). Within the valley, the Yellow-vented Bulbul (*Pycnonotus xanthopygos*) was the only frugivore observed dispersing the seeds of *P. acaciae*, a mistletoe native to the Middle East and Africa. The bulbuls swallowed the fruits whole and defecated seeds intact, with *P. acaciae* fruits accounting for a substantial proportion of the bulbul diet when the fruits were most abundant.

3.3.2 Africa

The ripe fruits of African Loranthaceae are 5 – 15 mm in length, with a thin but tough exocarp that is usually yellow – red or rarely blue in colour (Polhill 1989, Polhill and Wiens 1998). The primary dispersal agents are the tinkerbirds, *Pogoniulus* spp. (Capitonidae) which remove the exocarp of the fruit before swallowing it and subsequently regurgitate the seeds. Once regurgitated, the birds then wipe the seed off onto a branch with their bill where it can adhere (Polhill 1989, Roxburgh 2007). On the Nyika Plateau in Malawi, the Green-moustached tinkerbird *Pogoniulus leucomystax* is almost the only recorded disperser of seeds of several Loranthaceae species from the genera *Tapianthus*, *Phragmanthera*, *Englerina* and *Dendrophthoe* (Dowsett-Lemaire 1982, 1988). In younger patches of the forest where only one mistletoe species *Englerina* occurs, the tinkerbirds move in only temporarily to breed during the fruiting season (Dowsett-Lemaire 1982).

Within the Loskop Dam Nature Reserve of South Africa, 7 of 27 bird species present were observed eating Loranthaceae fruit, the most important of which was the yellowfronted tinker barbet *Pogoniulus chrysoconus* (Godschalk 1983b, Godschalk 1985). The tinker barbet was observed to consume 64 – 80 % of *Tapinanthus* and *Englerina* fruit. This species was considered to be the most behaviourally suited to effective dispersal of the seeds because single regurgitated seeds were wiped directly onto branches (Godschalk 1985). Of the other bird groups recorded as opportunistically eating the fruits of African Loranthaceae, the mousebirds (Coliidae) defecate the seeds, starlings (Sturnidae) and barbets (Lybiidae) regurgitate, whilst the tit (Paridae) and weaver birds (Ploceidae) peck flesh off the fruits (Godschalk 1983b, Roxburgh 2007). Mousebirds may be important for long-distance dispersal of seeds due to the fact that they have relatively long post-feeding flights and greater mobility than other species (Godschalk 1985).

3.4 Aims

Knowledge of the dispersal ecology of African mistletoes is based largely on observations of fruiting plants. The focus of this section of study is to investigate some aspects of the dispersal ecology of three West African mistletoe species, *Globimetula braunii*, *Agelanthus brunneus* and *Agelanthus djurensis* in an Afromontane forest habitat.

More specially, for each species I aim to:

1. Describe the morphological characteristics of the fruits
2. Investigate fruit ripening rates and dispersal efficiency

3.5 Methods and Materials

3.5.1 Description of fruits

Previous published records of the fruit characteristics of *Globimetula braunii*, *Agelanthus brunneus* and *A. djurensis* are limited. The ripe fruits of *G. braunii* are described as orange or red, 5 – 6 mm in length and 5 mm in width. The seed is also recorded as orange or red, 3 mm in length and 1.5 mm wide. The ripe fruits of *A. brunneus* are reddish in colour, smooth and obovoid to urceolate, 7 – 8 mm length and 4.5 – 5 mm width. The ripe fruits of *A. djurensis* are red or white, obovoid, 9 mm in length, 6 mm wide, and sparsely covered in small pimples, at least when dried (Keay 1958, Polhill and Wiens 1998).

The general appearance and colour was described for the unripe, ripe and overripe fruits of *G. braunii*, *A. brunneus* and *A. djurensis* at Ngel Nyaki. In 2008/09, fruit and seed size (maximum length and width) was measured using digital calipers (to the nearest 0.1 mm) for a total of 30 arbitrarily selected ripe *A. djurensis* fruits and 29 *G. braunii* fruits. *A. brunneus* fruits had not yet ripened so were not measured. Seeds and pulp were separated and sun dried for approximately 7 days, and subsequently weighed using digital scales (to the nearest 0.1 g). As the individual weights were too small to be registered accurately by the scales available for use at the field station, all fruit pulps, and all seeds were combined and each divided by the total sample size to obtain the average dry weights.

3.5.2 Fruit ripening rates and dispersal efficiency

2007/08 season

The methods used in this section largely follow those of Kelly et al (2004). To assess fruit ripening rates and dispersal efficiency, 1684 and 426 unripe fruits were mapped on ten *G. braunii* and four *A. brunneus* plants respectively. Fruits were initially selected by choosing 1-2 branches on each study plant bearing only unripe fruits and counting all fruits on these branches.

Fortnightly monitoring (census) commenced within one week of noticing the first ripe *G. braunii* fruits in March 2008. Monitoring of *A. brunneus* fruits started in April; approximately two weeks after the first *A. brunneus* fruits had begun to ripen. Monitoring of both species continued until

the end of the fruiting season when fewer than 5% of initial fruits remained on the branches. The number of unripe (green), ripe (red) and overripe (dark red/brown and wrinkled) fruits was recorded at each visit. To determine whether fruits that were missing from the branches had been dispersed or had fallen from the plant, a 1 m x 1 m piece of green curtain netting was suspended 0.5 - 1 m beneath the monitored branches on each plant using ties and wooden stakes. The number and state (unripe, ripe or overripe) of each whole fruit in the netting was recorded at every fortnightly visit and the net emptied.

2008/09 season

A total of 1151 unripe fruits were mapped on six *A. djurensis* plants. Fortnightly monitoring commenced within one week of noticing the first ripe *A. djurensis* fruits, in late January 2009 and continued until the end of March 2009 when monitoring had to cease for logistical reasons. At this time, around 17% of initial fruits remained on the branches. The same methods were used as in 2007/08, however this season, to check for loss of fallen fruits from the netting, another net was set up near three of the *A. djurensis* plants and 20 hand-picked fruits placed in each of the three nets at the first census. The proportion of these 20 fruits remaining at each subsequent fortnightly visit was recorded, and if any seeds were missing, these were replaced each time to maintain a known number of 20 seeds. The percentage of fruits retrieved at each census were calculated and averaged over all censuses to provide a correction factor for fruit loss from the netting (Kelly et al. 2004).

Analysis

To determine dispersal efficiency, the mean percentage of all initial fruits which were unripe, ripe and overripe at each census was calculated, along with the cumulative proportion of initial fruits which had fallen into the nets. After allowing for percentage losses from nets as described above, it was assumed that any missing fruits had been dispersed. The mean percentage of all fruits on the monitored branches in the ripe and overripe states was also calculated for each visit to then calculate the maximum percentage of fruits that remained on the branches in these states at any census during the fruiting season.

3.6 Results

3.6.1 Description of fruits

The fruits of *G. braunii* are green when unripe, and become redder as they ripen (Figs 3.1 and 3.2). *G. braunii* fruits were the largest of the three species, and notably larger at Ngel Nyaki than has been previously recorded for this species (see section 3.5.1 and Table 3.1) (Polhill and Wiens 1998). Dry seed weight was not obtained due to technical difficulties; however the fresh seed constituted almost 40% of the total fresh weight of the fruit (Table 3.1).



Fig 3.1: Ripening *Globimetula braunii* fruits



Fig 3.2: Ripening *Globimetula braunii* fruits. Note the one 'overripe' fruit (marked with white arrow)

The fruits of *A. brunneus* are also green when unripe, becoming red on ripening (Fig 3.3). Measurements were not obtained for *A. brunneus* fruits as the fruits did not ripen during the study period in 2008/09 when measurements for the other two species were taken.



Fig 3.3: Ripe *Agelanthus brunneus* fruits. Photo: Usman Usuf

A. djurensis fruits were green when unripe and became red on ripening (Fig. 3.4). The embryo was also green, completely surrounded by white viscid material and an outer orange fleshy layer (Fig 3.5). The seeds appeared to adhere to the substrate at one end (Fig 3.6). The fruits were smaller on average than those of *G. braunii* and percentage water content of the fruit pulp was higher. As in *G. braunii*, the seed comprised approximately 40% of the total fresh weight of the fruit (Table 3.1).



Fig 3.4: Ripe *Agelanthus djurensis* fruit



Fig 3.5: Open ripe *Agelanthus djurensis* fruit showing green embryo, orange flesh and white viscid material



Fig 3.6: Dispersed seeds of *Agelanthus djurensis* and one seedling.

Table 3.1: Summary of fruit characteristics of *Globimetula braunii* (n=29) and *Agelanthus djurensis* (n=30). Fruits of *Agelanthus brunneus* were not measured in this study as fruit did not ripen during the study period in 2009. Means \pm S.E are shown. Wet and dry weights presented are total weight of all samples divided by number of samples (see methods). –, no data.

	<i>G. braunii</i> (n=29)	<i>A. brunneus</i>	<i>A. djurensis</i> (n=30)
Fruit length (mm)	9.92 \pm 0.13	7 - 8.00 ¹	7.09 \pm 0.09
Fruit width (mm)	8.50 \pm 0.08	4.50 – 5 ¹	5.24 \pm 0.09
Seed length (mm)	5.99 \pm 0.05	-	4.18 \pm 0.12
Seed width (mm)	3.69 \pm 0.06	-	2.30 \pm 0.08
Wet pulp weight (g)	0.07	-	0.07
Wet seed weight (g)	0.04	-	0.05
Dry pulp weight (g)	0.03	-	0.02
Dry seed weight (g)	0.004*	-	0.005
Seed as % of total fruit weight	38.7%	-	43.2%
% water content of pulp	57.9%	-	71.0%
% water content of seed	-	-	90.6%
Ripe fruit colour	Red	Red	Red

¹ Polhill and Weins (1998)

* Actual dry weight not obtained, value shown is estimated using known % water of *A. djurensis*

3.6.2 Fruit ripening rates and dispersal efficiency

Globimetula braunii

In the 2007/08 season, a total of 1684 unripe fruit were mapped on 10 *G. braunii* plants (Fig 3.7). One *G. braunii* plant died two months after monitoring began leaving 1626 fruits on 9 *G. braunii* plants for monitoring. Fruiting of *G. braunii* lasted approximately 12 weeks, with the first fruits observed ripe in March 2008 and very few remaining unripe by the end of May 2008 (5% of 1684 monitored fruits) (Fig 3.8). A relatively low mean proportion of the *G. braunii* fruit crop were present ripe or overripe on branches at each fortnightly census (5% and 6% respectively) (Table 3.2). The highest proportion of ripe or overripe fruit seen at any one census was at the end of the fruiting season on 17th May 2008 when on average 10% of fruit on branches were ripe and 11% were overripe. This result suggests either fruits were removed rapidly once they ripened or ripened rapidly and fell into the nets overripe between censuses. On average, almost a third (31%) of a plants' total fruit crop had fallen undispersed into the net beneath the plant by the end

of the fruiting season (Table 3.2). The majority ($68\% \pm 18\%$ S.E) of fruits recorded in the nets at each census were overripe, with $21\% (\pm 14\%$ S.E) unripe and the remaining $11\% (\pm 5\%$ S.E) ripe. It is presumed that unripe and ripe fruits in the net were dislodged by foraging birds (Kelly et al, 2004). It is possible that the number of fruits fallen into the nets had initially been even higher, with some subsequently eaten by other animals or having undergone secondary dispersal from the fruit nets between censuses.

In the following 2008/09 season, ripening of *G. braunii* fruit was not observed until April and the majority of fruits were still unripe by end of May. It is not clear whether fruiting of *G. braunii* was early in 2007/08, or delayed in 2008/09.



Fig 3.7: Monitored *Globimetula braunii* fruits showing net below.

Agelanthus brunneus

In the 2007/08 season 426 fruits on four *A. brunneus* plants were mapped. The fruits of *A. brunneus* were first observed ripening around late March to early April 2008 and none of the 426 monitored fruits remained unripe by mid-June 2008. The duration of *A. brunneus* fruiting was approximately 10 weeks in 2008 (Fig 3.8). The mean proportion of the *A. brunneus* fruit crop which were present ripe or overripe on branches at each census was 13% and 7% respectively (Table 3.2). The highest proportion of ripe or overripe fruit recorded at any one census were observed at the end of the fruiting season, when on average 12% were overripe on 31 May 2008 and 100% of fruit on branches was ripe on 14 June 2008. On average only 8% of the initial fruit crop was found in the fruit net during the fruiting season (i.e. undispersed), suggesting relatively high dispersal efficiency for this species. All of the fruits found in the nets were overripe. It is possible that other fruits which had initially fallen into the nets had been eaten or undergone secondary dispersal by other animals.

In 2008/09, the fruits of *A. brunneus* remained unripe until the end of the study period in June 2009 and consequently no data was collected.

Agelanthus djurensis

In 2008/09, 1126 fruits were mapped on six *A. djurensis* plants. Fruiting lasted approximately 10 weeks. The first ripe fruits were observed in late January 2009, with 17% of the 1126 monitored fruits remaining unripe by the end of the monitoring period in March 2009 (Fig 3.8). A relatively low mean proportion of the *A. djurensis* fruit crop were present ripe or overripe on branches at each fortnightly census (8% and 6% respectively) (Table 3.2). As was observed for the other two mistletoe species, the proportion of ripe or overripe fruit on branches was highest towards the end of the fruiting season, when on average 22% of fruit was ripe and 19% was overripe. On average 8% of a plants' total fruit crop fell undispersed into the net beneath the plant during the fruiting season, all of which were overripe (Table 3.2). Of the fruits that were placed in fruit traps to test for subsequent predation or secondary dispersal by other animals, 96% ($\pm 10\%$ S.E) were recovered at the next census. This suggests that the numbers of fruits recorded within the fruit nets (at least for *A. djurensis*), were an accurate reflection of actual numbers of fruits fallen throughout the season.

Table 3.2: Percentage of ripe and overripe fruits on branches and dispersal efficiency for *Globimetula braunii*, *Agelanthus brunneus* and *Agelanthus djurensis*. Mean % (\pm S.E) ripe and overripe are averages across all censuses from within a week of first ripening (except for *A. brunneus* which was c.2 weeks later) until $\leq 5\%$ of fruits remained on branches (or 17% for *A. djurensis*). Total % fallen is mean cumulative for whole season.

	Mean % ripe	Range % ripe	Mean % overripe	Range % overripe	Total % fallen	Number of fruits
<i>G. braunii</i>	5.10 \pm 1.62	0.07 - 9.60	5.44 \pm 1.72	0.00 - 10.41	30.54	1626
<i>A. brunneus</i>	13.06 \pm 17.72	6.10 - 100	6.93 \pm 2.32	0.00 - 12.31	7.79	426
<i>A. djurensis</i>	7.81 \pm 3.68	1.44 - 21.80	6.29 \pm 3.68	0.00 - 18.48	8.43	1126

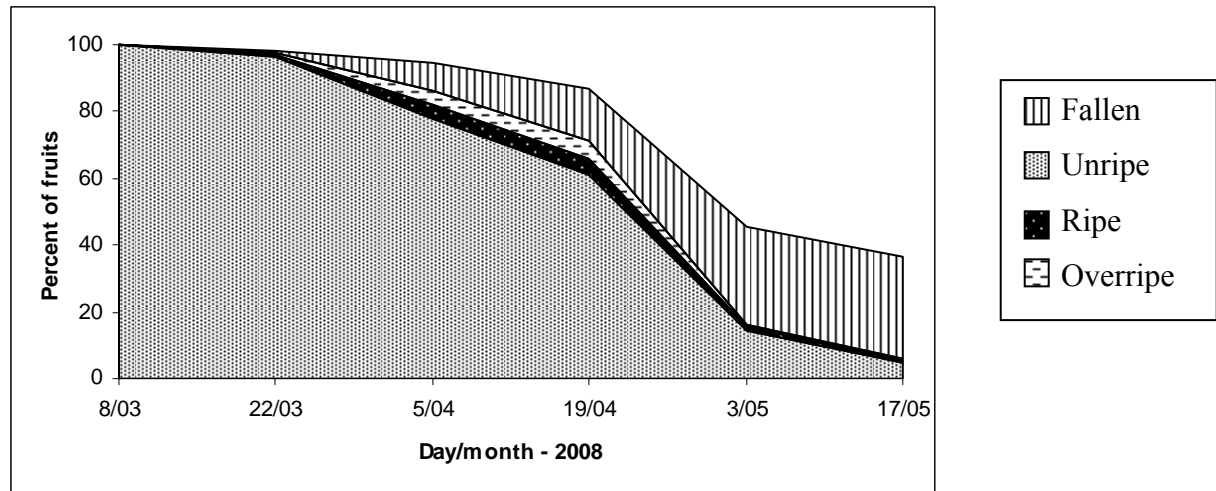
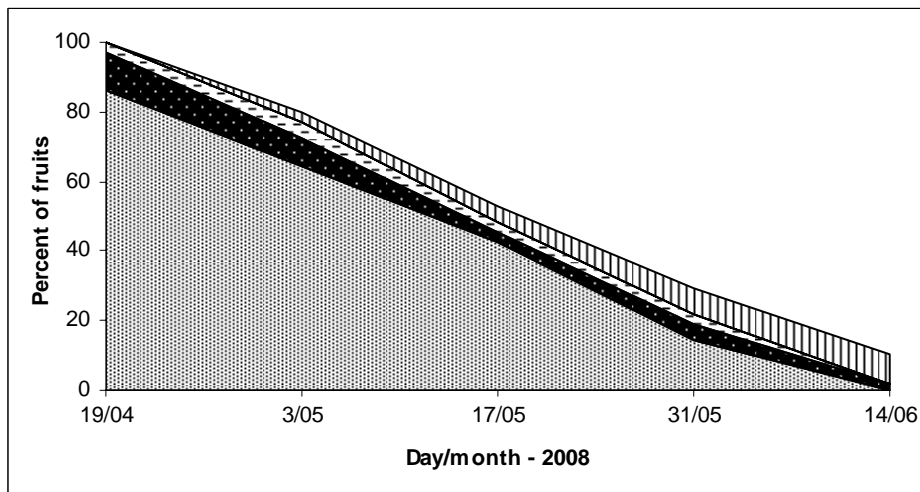
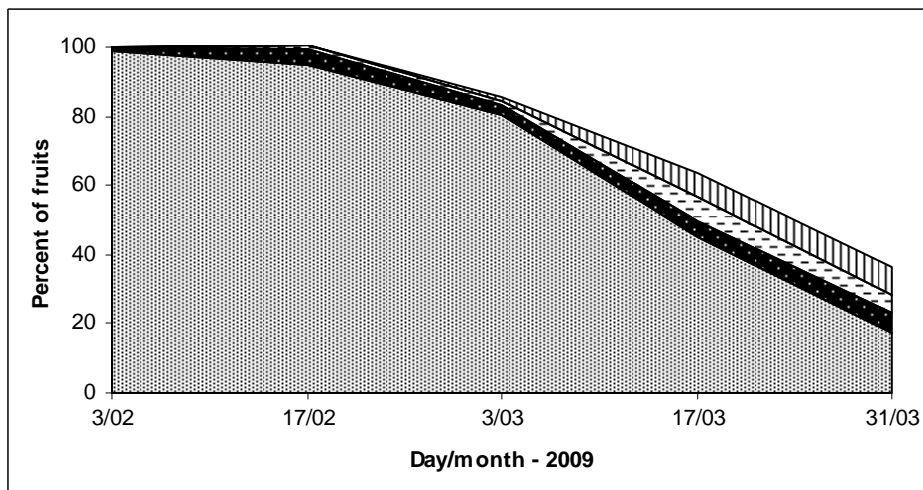
(a) Fruit ripening and removal for *G. braunii*(b) Fruit ripening and removal for *A. brunneus*(c) Fruit ripening and removal for *A. djurensis*

Fig 3.8: The proportion of initial fruits which were unripe, ripe and overripe at each census during fruiting for (a) *Globimetula braunii*, (b) *Agelanthus brunneus* and (c) *Agelanthus djurensis*. Values for “fallen” fruits are cumulative throughout the season.

3.7 Discussion

3.7.1 Fruit characteristics

Fruit and seed dimensions of the Ngel Nyaki mistletoes *G. braunii* and *A. djurensis* obtained in this study are within the range of dimensions recorded for other Loranthaceae worldwide (Ali 1931, Docters Van Leeuwen 1954, Kujit 1969, Bhatnagar and Johri 1983, Godschalk 1983a, Hoffmann et al. 1986, Reid 1986, Dowsett-Lemaire 1988, Polhill 1989, Ladley 1994, Martinez del Rio et al. 1995, Ladley and Kelly 1996, Lopez de Buen and Ornelas 1999, Amico and Aizen 2000). However, measurements varied from those recorded for these species by Polhill and Weins (1998) in other parts of Africa. Most notably, Polhill and Weins (1998) recorded the fruits of *G. braunii* as 5 – 6 mm in length and 5 mm in width, while in the present study they were on average 9.9 mm in length and 8.5 mm in width. Considerable intraspecific variation in fruit morphology has been recorded across a wide range of other species, so that this difference among sites is not unexpected given that such variation is fundamental to the principles of natural selection (Willson et al. 1990, Obeso and Herrera 1994, Jordano 1995b, Izhaki et al. 2002).

Percentage water within the fruit pulp of *G. braunii* and *A. djurensis* was also within the range previously recorded for Loranthaceae (Godschalk 1983b, Ladley 1994, Lopez De Buen and Ornelas 2001, Kelly et al. 2004). However percentage water within the seeds of *A. djurensis* (90%) was unexpectedly high, although previous records of seed water content for mistletoes are lacking in the literature. Further investigation of the wet and dry seed weights of these species would be valuable. The fresh seeds of *G. braunii* and *A. djurensis* constituted approximately 40% of the total wet weight of the seeds. Lopez de braun (2001) calculated a similar fruit to seed weight ratio in *Psittacanthus schiedeianus* where a single seed constituted 36.4% of the total fruit weight. New Zealand Loranthaceae have been shown to have highly variable seed to fruit weight ratios, with seed weight ranging from 15.89% to 73.68% of the total fruit weight for five species (Ladley and Kelly 1996).

The colour of ripe fruits was red for all three mistletoe species. Across Africa, red predominates as the most common colour for ripe Loranthaceae fruits. Of the 128 African Loranthaceae species where ripe fruit colour has been described, 77% have red or partly red fruit (Polhill and Wiens 1998). In the Neotropics, approximately 25% of bird-dispersed fruits are ripe when red, and in

Central Europe and Spain, around 40% (Herrera 2002). The particularly high frequency of red fruits amongst African Loranths may be attributed to a trait preference exhibited by their common primary dispersal agents, the tinkerbirds (*Pogoniulus* spp.). This is on the basis that plants which evolve traits attractive to dispersers will be visited more and thus gain greater dispersal efficiency and ultimately a higher fitness advantage. Evidence supporting fruit colour as an adaptive response to frugivore preference is contradictory. Although studies have shown a preference for certain fruit colours under controlled conditions (Puckey et al. 1996, Traveset and Willson 1998, Gervais et al. 1999, Giles and Lill 1999, Alves-Costa and Lopes 2001, Galetti et al. 2003, Honkavaara et al. 2004) evidence that such preferences translate into greater dispersal efficiency under natural conditions is limited and largely derived from studies of fruit colour polymorphisms (Traveset and Willson 1998, Bach and Kelly 2004, Whitney and Lister 2004, Tsujita et al. 2008). Furthermore, the bird-dispersed fruits of the New Zealand Lorantheae *Peraxilla tetrapetala* ripen a dull-green colour and these fruits have been shown to undergo adequate biotic dispersal (Kelly et al. 2004). Other selective pressures such as defence against insect or microbial attack, the deterrence of poor dispersal agents, physiological factors, phylogenetic history or indirect evolution by selection acting on correlated characters may all contribute to explaining fruit colour variation amongst fruiting plants (Willson and Whelan 1990, Herrera 2002).

3.7.2 Fruit ripening rates and dispersal efficiency

Few ripe or overripe fruits were present on branches at any one census (5 – 13%) for all three mistletoes, *G. braunii*, *A. brunneus* and *A. djurensis* (Table 3.2). In the New Zealand Lorantheae *Peraxilla tetrapetala* and *Alepis flavida*, the proportion of ripe or overripe fruits present on branches at any time ranged from < 1% to 15% across four years (Kelly et al. 2004). A low number of ripe or overripe fruits present on branches suggests either that i) dispersers dispersed fruits at a rapid rate upon ripening, or that ii) fruits ripened and fell from the plants in between censuses. If the latter scenario was the case, high numbers of ripe to overripe fruits would have been found in the fruit traps beneath the plants. However, this was not the case because the cumulative proportion of fruit crop which fell undispersed into the nets beneath plants was low for both *A. brunneus* (7.8%) and *A. djurensis* (8.4%). It is possible that fruits which fell undispersed into the net were subsequently eaten, or taken by secondary dispersers. Several bird species have been recorded as gleaning fallen *Tapinanthus globiferus* (Lorantheae)

fruits from the ground following rain storms in Southern Nigeria including three Doves species *Streptopelia semitorquata*, *S. senegalensis* and *Turtur abyssinicus* (Bright et al. 2001). However, the high recovery rate (96%) of a known number of fruits placed in fruit traps beside three *A. djurensis* plants suggests that the numbers of fruits recorded within nets beneath plants, (at least for *A. djurensis*), was an accurate reflection of actual numbers of fruit fallen.

In contrast to the two *Agelanthus* spp., almost a third (30.5%) of the total fruit crop of *G. braunii* fell into the nets. Therefore, dispersal efficiency appeared to be relatively lower for this species. All of the fruits found in nets were overripe for the two *Agelanthus* spp., as were the majority (68%) for *G. braunii*. This suggests that either the fruits became ripe in the nets, or that fruits over-ripened on branches prior to falling into the nets. As 5 – 7% of fruits on branches were observed to be overripe at any one census (Table 3.2), it is most likely that fruits fell into the net overripe.

Wider studies assessing the proportion of fruit crop removed by vertebrates reveal fruit removal to be highly variable both within and between fruiting species (Herrera 1984, Jordano 1987a, Murray 1987, French and Westoby 1992, Sallabanks 1992, Masaki et al. 1994, McCarty et al. 2002, Kwit et al. 2004). Fragmentation has been shown to have significant negative impacts on fruit removal and seedling recruitment in the South American Loranthaceae *Tristerix corymbosus* (Rodriguez-Cabal et al. 2007). There in fragments, fruit removal was between 30 – 50%, whereas in continuous forest it was consistently higher and ranged from 60 – 90%. These differences were attributed to the decline in abundance of the mistletoes only known dispersal agent, the endemic marsupial *Dromiciops gliroides* within fragmented forest (Rodriguez-Cabal et al. 2007). The proportion of undispersed fruit crop in New Zealand mistletoes has been reported to range from 0 – 26.9% for five Loranthaceae species across several fruiting seasons (Ladley and Kelly 1996, Kelly et al. 2004). Kelly et al. (2004) proposed that high predation events associated with masting of the mistletoes' host tree *Nothofagus solandri* may have limited densities of the mistletoes primary avian disperser, the bellbird *Anthornis melanura* (Meliphagidae) and contributed to dispersal limitation in at least one of the five species.

In this study, a variety of factors may have contributed to the lower dispersal efficiency experienced by *G. braunii* relative to the *Agelanthus* spp. One possibility is that factors affecting the density or visitation rates of dispersal agents to *G. braunii* may have limited dispersal efficiency in this species. Also, a preference for the fruits of *Agelanthus* spp. or other local

species fruiting concurrently, may have attracted dispersers away from the fruits of *G. braunii*. However, the fruits of *G. braunii* are large relative to those of the *Agelanthus* spp., and both the seed-pulp ratio and pulp water content are lower in *G. braunii*, all traits which may suggest the fruits of this species might be more attractive to frugivores. Without nutritional and mineral analysis of the fruits of all three species, further comparisons of morphological traits between species cannot be drawn. Support for the role of nutritional value in determining fruit-removal efficiency is mixed. Some report fruit removal to be primarily dependent on fruit nutritional traits (Izhaki 2002). Others stress there is often no relationship between pulp nutritional value and fruit removal, but that plant phylogeny at the genus level and above largely explains nutritional concentration in the absence of an adaptive response to dispersers (Herrera 1984, Jordano 1989, 1995a, Herrera 2002). The presence of secondary metabolites in fruits may further confound assessment of the adaptive role of fruit nutritional value and although not addressed in the present study, has important implications for fruit removal efficiency in general (Cipollini and Levey 1997).

Fruit crop size has been proposed to affect fruit removal, with plants bearing large fruit crops experiencing greater dispersal efficiency (Snow 1971, Howe and Estabrook 1977). However, many studies have shown no significant relationship between fruit crop size and the proportion of fruits removed (Davidar and Morton 1986, Murray 1987, Izhaki 2002, Saracco et al. 2005). Crop size of the Ngel Nyaki mistletoes was not assessed as fruits were counted only on one or two branches of each plant. However given that fruit set under natural conditions was only 6% in 2007/08, the year that fruit removal was monitored, crop size may have been limited. Natural fruit set of *A. brunneus* was also low in 2007/08 however, with only 3% fruit set under natural conditions, yet dispersal efficiency was found to be high for this species. Other variables such as the local fruiting environment and proximity to neighbouring conspecifics may have contributed to the differential dispersal efficiency between the two genera of Ngel Nyaki mistletoes and should be considered in future studies (Levey et al. 1984, Sargent 1990, Saracco et al. 2005, Carlo and Morales 2008).

3.7.3 Limitations of this study

This study was limited to plants located along the forest edge and consequently fruit removal may have been influenced by edge effects (Murcia 1995). Previous studies have shown plants located along the forest edge experience higher dispersal efficiency than those located in the forest interior, (Galetti et al. 2003, Bach and Kelly 2004) which may be attributed to a higher abundance of dispersers at the forest edge (Restrepo et al. 1999, Dale et al. 2000). However, work on the New Zealand mistletoe *Peraxilla tetrapetala* has shown no significant edge effects on the percentage of overripe fruits present on branches (Kelly et al. 2000). In Africa, tinkerbirds (*Pogoniulus* spp.) have been shown to exhibit no significant increase in density towards the forest edge, although at least one species, *P. scolopaceus*, exhibits a strong tendency to decrease in numbers towards the forest interior (Dale et al. 2000).

It is possible that the fruit nets beneath the mistletoe plants may have detracted some frugivorous visitors from the plants. Efforts were made to decrease the conspicuousness of the nets to potential visitors by using green-coloured netting where available. The high dispersal efficiency found in at least the two *Agelanthus* spp. indicates that even if some dispersers were scared off by the nets, this did not have a significant bearing on overall dispersal efficiency.

This study only assessed the quantity component of dispersal and no assessments were made of dispersal quality, such as treatment by dispersers, place and mode of deposition and percentage germination (Schupp 1993). However, it is likely that dispersal efficiency is highly correlated with germinability of seeds, for two reasons. Firstly, because the Ngel Nyaki mistletoes are aerial stem parasites, any undispersed seeds which fall to the ground are unable to establish beneath the parent plant. Secondly, previous studies have shown that mistletoe seed germination is triggered by removal of the exocarpic layer of the fruit, which under natural conditions is performed by dispersal agents (Calder 1983, Ladley and Kelly 1996). I enclosed 30 *A. djurensis* fruits within bags in 2008/09 to observe whether germination could occur within the intact fruits. None of these showed any signs of germinating within the bags throughout the fruiting season, and all subsequently over-ripened and fell into the bag where they dried and shriveled.

3.7.4 Conclusions

The overall mutualist service provided by dispersers appeared to be effective in terms of both the rate and proportion of fruits dispersed. The fruits of all three mistletoe species appeared to be removed rapidly from plants as they ripened, with few ripe or overripe fruits present on the branches at any time. Dispersal efficiency, or the total proportion of fruit crop removed across the fruiting season, was also very high (>90%) for the *Agelanthus* spp. but lower in *G. braunii*, for which almost a third of the total fruit crop was recorded undispersed in fruit nets beneath plants. Further investigation of individual fruit ripening rates when access by dispersers is prevented may help elucidate this seemingly disparate result between the low percentage of ripe and overripe fruits present and the high proportion of undispersed fruit crop for *G. braunii*.

It is unclear why the *Agelanthus* spp. experienced higher dispersal efficiency relative to *G. braunii*, although a variety of factors, including fruit traits, crop size, fruiting environment and neighbourhood effects may have contributed to the observed differences between the two genera. The primary dispersal agents of each species still require identification, and assessments of dispersal quality should be undertaken. The dispersal ecology of the Ngel Nyaki mistletoes offers much potential for further research.

4.1 General discussion

The overall objective of this study was to investigate the importance of plant-animal mutualisms to the reproductive ecology of three West African mistletoe species, *Globimetula braunii*, *Agelanthus brunneus* and *A. djurensis*, in Ngel Nyaki Forest Reserve, Nigeria. In this discussion I will first review the major findings of my research with respect to the pollination and dispersal ecology of the plants. I will identify potential linkages between the two mutualisms, and discuss the relative role and effectiveness of animal mutualists within and between the two mistletoe genera studied. Secondly, the potential for coevolution between the African Loranthaceae and their pollination mutualists will be discussed. Thirdly, I will explore the potential implications of the interactions revealed in this study for West African montane forest ecosystems.

4.1.1 Reproductive mutualisms

This investigation reveals that animal mutualists are essential to the overall reproductive output of all three mistletoe species although several key differences in reproductive ecology were found between the two genera. The flowers of *G. braunii* were able to self-open in the absence of pollinators, whereas pollinators were essential to the flower opening mechanism of both *Agelanthus* spp. Insects, identified as a small social wasp species (Vespinae) on *A. djurensis*, were found to open the flowers of *Agelanthus* spp. as effectively as sunbirds. However, flower-opening ability did not translate directly into pollination effectiveness, as insect access alone did not result in significantly higher fruit set than that observed under the bagged condition. Therefore, successful pollination in both genera hinged solely on a single guild of 3-4 closely related sunbird species.

Bond (1994) proposed a framework by which to assess plant attributes against extinction risk. As regards mutualisms, Bond (1994) suggested three points should be considered when attempting to evaluate the importance of reproductive mutualisms: the probability of a mutualism failing, reproductive dependence on the mutualism and demographic dependence on seeds. The probability of a mutualism failing is directly related to the specificity of the mutualism. Although in this study each mistletoe species was visited by at least three species of sunbird, the fact that the sunbirds are from two closely related genera increases the specificity, and thus probability of the mutualism failing. Similarly, if the mistletoes' dispersal mutualists in Ngel Nyaki are found to

belong to a single taxon, (as might be expected because tinkerbirds (*Pogoniulus* spp.) are thought to be the primary agents of dispersal for African Loranthaceae (Polhill 1989)), then the probability of mutualism failure will be higher. The extent to which mutualism failure will affect the reproductive output of a plant is dictated by the degree of reproductive dependence on the mutualism (Bond 1994).

All three of the mistletoe species studied at Ngel Nyaki were found to be highly self compatible. However, there was no significant evidence for autonomous seed production which indicates reproductive dependence on mutualists. Only *G. braunii* was found to be pollen limited during this study. One possible explanation proposed for the limited pollination service experienced by *G. braunii* was that during peak flowering in December, sunbirds were preferentially visiting the flowers of a large endemic tree *Anthonothea noldeii* which was also in full flower at this time. Large numbers of sunbirds were observed to be foraging on *A. noldeii* around the forest edge (pers. obs). Another possibility is that *G. braunii* serves as an early indicator of ecological disruption, which may ultimately have consequences on all three mistletoe species over time. Even where pollination service was found to be adequate, as was the case for the *Agelanthus* species, successful reproduction is not strictly complete until the seedlings become established. Germination and establishment in Loranthaceae is reliant on dispersal agents due to both the inability of seeds to germinate within intact fruits and for undispersed seeds to establish beneath the parent plant (Calder 1983, Ladley and Kelly 1996).

The overall mutualist service provided by dispersers during this study appeared to be effective in terms of both the rate and proportion of mistletoe fruits dispersed. However, dispersal efficiency or the total proportion of fruit crop removed across the fruiting season was relatively lower in *G. braunii* compared with the *Agelanthus* species.

Demographic dependence on seeds was inferred to be high for the mistletoes due to the lack of asexual reproduction or vegetative spread between host trees and the relatively short life span of mistletoes (up to several decades: Roger Pohill, pers. comm.) in comparison to other woody plants. If seedling densities exceed the availability of establishment sites and post-dispersal density-dependent mortality is high, then dependence on seeds may be lower given that population growth will be largely insensitive to seed production. As discussed in Section 3.3.1, evidence for density-dependent mortality amongst mistletoe seedlings is mixed, although both

pollen and seed limitation has been found within at least two populations of the New Zealand Loranthaceae *Peraxilla tetrapetala* (Kelly et al. 2007).

It is interesting to consider that both the pollination and dispersal mutualism of *G. braunii* appeared limited by mutualist service. This result may not be unexpected given the potential mechanisms for linkage between these two processes. At the level of the individual plant, crop size is directly associated with pollination success (Jordano and Schupp 2000, Corlett 2007) and as previously discussed (see section 3.7), increased fruit crop size has been proposed to result in greater dispersal efficiency (Snow 1971, Howe and Estabrook 1977). The two mutualisms are also linked at population level as seed dispersal mechanisms may influence plant densities and distances between conspecifics, in turn affecting pollinator behaviour (Feinsinger et al. 1991, Corlett 2007). For example, Feinsinger et al. (1991) showed that by artificially increasing local intraspecific plant densities, hummingbird visitation rates increased significantly, as did the number of pollen tubes in styles. For the self-incompatible species studied, a significant increase in seed output per flower with increasing density was also found. Further research into population structure and density of the Ngel Nyaki mistletoes may contribute further explanation to the differences in reproductive mutualisms found in this study, particularly between the two genera.

4.1.2 Coevolution

The Loranthaceae diverged as a distinct clade within the order Santalales some 81 million years ago. They first evolved on the Gondwanan landmass and underwent huge dispersal throughout Gondwana during the Cretaceous period (Barlow 1983, Vidal-Russell and Nickrent 2008b). Subsequent to this mass dispersal, the family appears to have undergone major radiation during the Oligocene (34 – 23 million years ago) (Vidal-Russell and Nickrent 2008b). The African Loranthaceae derive from Asia and represent a large isolated stock of 21 specialised genera, the majority (90%) of which are endemic to Africa, or Africa and the Arabian peninsula (Polhill 1989, Polhill and Wiens 1998). The extensive adaptive radiation of African Loranthaceae has been linked to two foundations, first, the widespread development of seasonally dry savannah habitat during the Miocene (23 – 5 million years ago) and second, interaction with primitive bird pollinators belonging to a group of Oscine passerines which dispersed into Africa from Australasia during the Oligocene (Polhill 1989, Barker et al. 2004, Vidal-Russell and Nickrent 2008a). The sunbirds (Nectarinidae) belong to this Oscine group and are the principal nectar-

feeding birds of Africa (Polhill and Wiens 1998). The sunbirds clearly share a long evolutionary history with the African Loranthaceae with both groups experiencing simultaneous radiation throughout Africa during the Oligocene. It is from this foundation that a case for the close association between the sunbirds and Loranthaceae, resulting from reciprocal evolutionary change can be explored.

This process of reciprocal evolutionary change, driven by natural selection, is widely known as ‘coevolution’ (Thompson 2005). Jansen (1980), more specifically defines coevolution as ‘an evolutionary change in a trait of the individuals in one population in response to a trait of the individuals of a second population, followed by an evolutionary response by the second population to the change in the first’. Coevolution between broad groups or ‘guilds’ of taxa, as opposed to pairwise interactions between species, is described as ‘diffuse’ coevolution (Janzen 1980). Obtaining evidence to evaluate the likelihood of coevolution as the shaping force behind an interaction involves making assessments at a variety of levels including across geographical, phylogenetic and species to multispecies networks (Thompson 2005).

The sunbirds are clearly well adapted to mistletoe foraging, with their long decurved bills and tube-like tongues. Optimal foraging theory assumes that foraging efficiency is directly associated with animal fitness, given that animals that are able to achieve higher net energy gain are most likely to survive and reproduce more offspring (Pyke 1984, Lemon 1991). Where variation in feeding apparatus significantly affects foraging efficiency, natural selection should act upon this variation leading ultimately to more specialised morphological traits which optimise foraging efficiency. In the case of the sunbirds, variation in tongue and bill morphology has been shown to significantly affect foraging time and the volume of nectar extracted from individual flowers with long corollas (Schlamowitz et al. 1976, Gill and Wolf 1978).

With regard to fitness benefits to the mistletoes, this study revealed significant variation in mistletoe reproductive output as an outcome of interaction with the sunbirds. Results from the exclusion experiments showed that seed production by the mistletoes was almost entirely reliant on the pollination service provided by 3-4 sunbird species. Specialised flower development, particularly within *Agelanthus* species appears to have evolved in response to selection for restricted access to all but the most effective sunbird pollinators. These specializations include a concealed nectar reward inside the mature bud, a long floral tube, a complex explosive flower

opening mechanism entirely reliant on pollinators in *Agelanthus* spp. and highly refined morphological and colour signaling both pre- and post-anthesis.

Thompson (1994) refers to the concept of attracting effective mutualists but excluding ineffective visitors and cheats as a ‘selective sieve’. In the present study, it was revealed that a small species of social wasp in the subfamily Vespinae was capable of opening flowers of the two *Agelanthus* species in this study, thereby passing through the selective sieve of *Agelanthus*. The wasps appeared to be harvesting pollen from the anthers and results of exclusion experiments revealed that the wasps did not significantly increase reproductive output in *Agelanthus* above that recorded when all pollinators were excluded. Pollen consumption reduces male fitness of the host plant due to a reduction in the pollen load available for dispersal to the stigmas of other plants in the population (Hargreaves et al. 2009). It is therefore expected that over time, *Agelanthus* should evolve structural defence against the wasps to prevent them from prying apart the corolla lobes as observed in this study. The strength of selection for the evolution of such defence should rest upon the loss of fitness *Agelanthus* experiences as a result of the pollen harvesting versus the fitness advantage of evolving such defence. It is of particular interest that a similar interaction has been documented between ornithophilous New Zealand Loranthaceae and small native bees of the genera *Hylaeus* and *Leioproctus* (Kelly et al. 1996, Robertson et al. 2005) where it was found one species (*Peraxilla colensoi*) has stiffer buds that the insects are unable to open. The Vespinae wasps at Ngel Nyaki appeared to be prising open the explosive mistletoe flowers in a similar manner to the New Zealand bees, making this discovery both a good example of parallel evolution and a reminder that specialisation over long evolutionary periods may be difficult to maintain (Thompson 2005).

At Ngel Nyaki, sunbirds do not feed exclusively on mistletoe flowers and other plant species, such as *Anthonothea noldeii*, have been observed to receive heavy sunbird visitation during peak flowering (pers. obs). Furthermore, on the Nyika Plateau in Malawi, the flowers of 40 plant species are visited by sunbirds, over half of which are also visited by insects (Dowsett-Lemaire 1989). Consequently, this asymmetry in the mistletoe-sunbird interaction contradicts conventional interpretation of the definition of coevolution, that is, as a pair-wise strictly reciprocal relationship between two populations. However, asymmetry and multispecific interaction does not necessarily negate coevolution in the diffuse sense (Thompson 1994). Thompson (1994, 2005) expanded on the definition of diffuse coevolution acknowledging that

selective pressures on traits are exerted by an array of species groups which converge on the core traits of a mutualism, as opposed to specialisation on other species directly. This convergence or specialisation on traits is receptive to change, both on a temporal and geographical scale.

For the mistletoe-sunbird interaction, asymmetry may be partly explained if in some communities, mistletoes provide a temporally vital resource for the sunbirds when other food sources are limited (Kirkup 1984). Although the strength and specialisation of the interaction may not be constant, selective pressure for coevolution between mistletoes and sunbirds may be high, if the fitness of both groups is disproportionately affected by the pollination interaction. Furthermore, in considering the structure of the association in a geographic context, interactions may be subject to reciprocal selection within only some local communities, in what have been termed 'coevolutionary hotspots' (Thompson 2005). Coadaptation may be maintained amongst these communities however, through gene-flow and meta-community dynamics.

On a geographical scale, African Lorantheae exhibit strong endemism, with approximately 70% of species confined to one or more of the floristic regions (phytochoria) defined by White (1983) (Polhill 1989). Selective pressures resulting from interspecific competition for nectar in more mesic and species-rich habitats (such as evergreen bushland, woodland and forest) has been proposed as a foundation for radiation within the African Loranthaceae by precipitating a trend towards increased concealment of nectar (Kirkup 1993). This is on the basis that more complex opening mechanisms obviate the need for territorial defence by sunbirds by creating an exclusive resource for species which can open them. In the more open dry country habitat where biodiversity is relatively lower, competition for nectar is proposed to be lower and a less specialised opening mechanism reflects reduced selection pressure for specialisation in these habitats (White 1983, Polhill 1989, Kirkup 1993). Convergence in gross morphology amongst bird-pollinated African Lorantheae genera, despite underlying structural and tissue differences, is thought to reflect strong selection by a common pollinating group, the sunbirds (Kirkup 1993).

The potential period of interaction between sunbirds and the African Lorantheae spans many millions of years. There is strong mutualistic congruency in traits between the two groups and a mutual fitness advantage associated with their interaction. However, given that the mistletoe-sunbird interaction appears to be largely asymmetric and multispecific, coevolution *sensu stricto* seems unlikely. Diffuse coevolution between local guilds of mistletoes and sunbirds seems

conceivable, and this close association appears to have at least contributed to the extensive adaptive radiation of African Loranthaceae.

4.1.3 Implications of mistletoe mutualism failure for West African montane forests

It is now widely recognised that breakdown of a reproductive mutualism may affect not only species-pairs directly involved in the interaction, but an entire network of species linked within a web of interactions (Bond 1994, Kearns et al. 1998, Memmott 1999, Bascompte and Jordano 2007). This may lead to cascading effects throughout an entire ecosystem, particularly when the species involved has a large and disproportionate impact within a community or ecosystem relative to its abundance (Paine 1969). Watson (2001) proposed that mistletoes function as a keystone resource in many forests and woodlands worldwide. This is on the basis that species from 97 vertebrate families have been recorded consuming mistletoe, the majority of which (over 80%) were recorded feeding on nectar and/or fruits. Furthermore, 43 families of birds and 7 families of mammals have been recorded using mistletoes as nesting sites. Due to the high incidence of these and other important mistletoe-animal interactions, loss of mistletoes from a forest ecosystem may affect local community dynamics and even species richness.

Across Africa, mistletoes are considered a highly favoured food source for a variety of large herbivores including gorilla (*Gorilla gorilla*) and elephants (*Loxodonta Africana*) (D'Arcy 1985, Polhill and Wiens 1998). This preference for mistletoe foliage may be attributed to a general lack of chemical defence in the leaves (Polhill and Wiens 1998) and higher levels of nutrients (Watson 2001). The importance of mistletoe foliage to the diet of primates at Ngel Nyaki is unknown, however tantulus monkeys (*Ceropithecus aethiops*) have been observed eating the leaves (Musa Amadu, pers. comm.).

The fruits of African mistletoes are dispersed primarily by tinkerbirds (*Pogoniulus* spp.) (Dowsett-Lemaire 1982, 1988, Godschalk 1983). On the Nyika Plateau of Malawi, several species of mistletoe fruit successively throughout the year and comprise a substantial component of the tinkerbird diet (Dowsett-Lemaire 1982, 1988). In patches of habitat where mistletoe species were scarce, tinkerbirds were recorded to be present only during the fruiting season when they moved in to breed. Although mistletoe fruits are generally bird-dispersed, chimpanzees (*Pan troglodytes*) have been recorded eating *Agelanthus brunneus* fruits in the Kahuzi montane forest of the Democratic Republic of Congo (Basabose 2002). The abundant nectar produced by

mistletoe flowers also provides an important energy source for their primary African pollinators, the sunbirds (Polhill and Wiens 1998).

Mistletoe mutualisms, and the potential importance of these mutualisms within the web of interactions in West African montane forests has, until now, been largely overlooked. Kirkup (1984) made observations of avian visitors to two Loranthaceous mistletoe species (*Tapinanthus bangwensis* and *Globimetula braunii*) in open bushland along the edges of Afromontane forest in the Bamenda Highlands, Cameroon. Here, three species of sunbirds were documented as the only pollinators of these mistletoes. Kirkup (1984) proposed that in certain areas, *G. braunii* may gain some assurance of visitation during flowering by being the only food source available for the sunbirds, after which time the birds migrate to other areas.

The present study revealed a specialised interaction between the Ngel Nyaki mistletoes and their avian pollinators, the sunbirds. Although insects visited the flowers of two of the three mistletoe species, reproduction was almost entirely dependent on 3-4 species of sunbird. Whilst the importance of the mistletoes as a resource for the sunbirds at Ngel Nyaki remains unknown, they may contribute an important resource to the annual feeding cycle of the sunbirds (Kirkup 1984, Dowsett-Lemaire 1989). Furthermore, sunbirds have been observed pollinating several different tree species within Ngel Nyaki montane forest (pers. obs.), and therefore a breakdown of the mistletoe-sunbird mutualism, or the mechanisms behind this breakdown, may have cascading effects on other plant species also reproductively reliant on the sunbirds. For dispersal, although the primary dispersers remain unknown, the same implications of mutualism breakdown are applicable. This is because the dispersal mechanism of mistletoe is also specialised and seed germination and establishment is entirely dependent on dispersal agents (Lamont 1983, Ladley and Kelly 1996).

Some postulate that species loss within ecosystems may not necessarily result in decline of the ecosystem process or service that species provided if other species consequently increase and functionally replace the species lost (Walker 1992). Although, as increased susceptibility to extinction is often associated with specialisation (Bond 1994), the potential for functionally equivalent species to compensate for species lost may in reality be somewhat limited (Sekercioglu et al. 2004, Kirika et al. 2008). In West African montane forests, it is possible that with the loss of sunbirds, another species may compensate as mistletoe pollinators. In West Africa, the only other avian nectar-feeder, and thus likely candidate for such compensation is the

white-eye (Zosteropidae). However, African white-eyes appear unable to open complicated flowers, such as those of many Loranthaceous mistletoes (Polhill 1989).

At Ngel Nyaki, the reproductive mutualisms of *Agelanthus* spp. appeared to be fully functional, with no evidence for pollen limitation or limited dispersal efficiency. However, this was not the case for *Globimetula braunii*. It is possible that the mutualism limitation revealed in *G. braunii* provides an early ecological indicator of change, which may have consequences for all three mistletoe species and their interactions over time. Given that Ngel Nyaki Reserve is comprised of remnant forest fragments, surrounded by heavily grazed and burnt open grassland and savannah, ecological disruption as a consequence of anthropogenic change is not unexpected (Chapman and Chapman 2001, Chapman et al. 2004). These threats are not unique to Ngel Nyaki, but are shared by many forests within the West African regional mountain system and are correlated with burgeoning population densities (Cheek et al. 2000, Chapman and Chapman 2001, Maisels et al. 2001, Chapman et al. 2004, Burgess et al. 2007). In the Kilum-Ijim montane forest of Cameroon, the red data list mistletoe *Tapinanthus letouzeyi* is threatened with extinction by changing land use for agriculture and the felling of host trees for firewood (Cheek et al. 2000). Disruption to mistletoe mutualisms may serve as an early indicator of the ultimate problems confronting West African montane forests in general. Without stricter enforcement of laws protecting these areas, and active involvement of local communities in management, the future of these forests remains tenuous.

Mistletoes are not only valuable within the West African montane forest ecosystem for their ecological value, but also for the medicinal properties they harbour (see also Sections 1.3.2 and 1.4). These properties are recognised on both a regional and international scale (Polhill and Wiens 1998, Olagunju et al. 1999, Fred-Jaiyesimi et al. 2008, Ie and Zam 2008). The loss of mistletoes from these forests may thus mean a loss of the properties and services they provide, the majority of which are yet to be realised.

Several mistletoe species have become serious weeds on economically important plantation crops in West Africa (Polhill and Wiens 1998, Begho et al. 2001, Bright et al. 2001). However, the perception that mistletoes are solely weeds throughout the region needs to be challenged. In highly modified environments, such as plantations, mistletoes may prosper under artificially intensified light and water regimes. However, under natural conditions, browsing by mammals, and outshading may keep mistletoes in check within the forest ecosystem. Lessons from the

management of New Zealand and Australian mistletoes suggest that understanding the regulators of mistletoe abundance and distribution, and the factors which modify these regulators, is necessary to manage both over and under-abundance of mistletoes within forest ecosystems (Norton and Reid 1997). For example, fragmentation may lead to an initial increase in mistletoe abundance along forest edges (Burgess et al. 2006), however reduced abundance of mistletoe mutualists in fragments may lead to an ultimate loss of mistletoes from heavily fragmented areas, once ecological thresholds are reached (Norton et al. 1995, Norton and Reid 1997). Furthermore, other factors such as an increase or decrease in herbivore abundance, altered light, water and nutrient regimes and changed abundance of flower, fruit and seed predators may all interact in disturbed landscapes to precipitate an under or over abundance of mistletoes within the ecosystem (Norton and Reid 1997, Kelly et al. 2008). A holistic approach to ecosystem management should encompass all of these factors by addressing the ultimate causes of changing mistletoe abundance.

I recommend that the population structure and density of all mistletoe species within the Ngel Nyaki reserve be recorded, followed by regular surveying to monitor trends over time. Mistletoes have high value both intrinsically and as a resource not only within Ngel Nyaki forest reserve, but across all West African montane forests where they occur. Further study of mistletoes across this region is strongly encouraged, and the recognition that mistletoes are an important component of these forest ecosystems should be promoted. Controlled early burning away from the forest edge is helping to prevent fires encroaching into the forest, and fencing of grassland areas in some areas within the reserve is facilitating forest regeneration at those localities (Chapman and Chapman 2001, Chapman et al. 2004, Beck and Chapman 2008). These actions may directly benefit the mistletoes, which are often found growing most abundantly along the forest edge and thus are particularly exposed to such impacts (Polhill 1989). Ultimately however, the future of the mistletoes and their web of interacting species depends upon the support of local communities, and the ability and willingness of local governments to enforce greater restrictions protecting West African montane forest habitat from further degradation.

References

- Aizen, M. A. 2003. Influences of animal pollination and seed dispersal on winter flowering in a temperate mistletoe. *Ecology* **84**:2613-2627.
- Aizen, M. A. 2005. Breeding system of *Tristerix corymbosus* (Loranthaceae), a winter-flowering mistletoe from the southern Andes. *Australian Journal of Botany* **53**:357-361.
- Ali, S. A. 1931. The role of sunbirds and flower-peckers in the propagation and distribution of the tree-parasite, *Loranthus longiflorus* Dest., in the Konkan (W.India). *Journal of the Bombay Natural History Society* **35**:144-149.
- Ali, S. A. 1932. Flower-birds and bird-flowers in India. *Journal of the Bombay Natural History Society* **35**:573-605.
- Alves-Costa, C. P. and A. V. Lopes. 2001. Using artificial fruits to evaluate fruit selection by birds in the field. *Biotropica* **33**:713.
- Amico, G. and M. A. Aizen. 2000. Mistletoe seed dispersal by a marsupial. *Nature* **408**:929-930.
- Ashman, T. L., and D. J. Schoen. 1996. Floral longevity: fitness consequences and resource costs. Pages 112-139 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral biology: studies on floral evolution in animal-pollinated plants*. Chapman and Hall, New York.
- Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**:2408-2421.
- Azpeitia, F. and C. Lara. 2006. Reproductive biology and pollination of the parasitic plant *Psittacanthus calyculatus* (Loranthaceae) in central Mexico. *Journal of the Torrey Botanical Society* **133**:429-438.
- Bach, C. E. and D. Kelly. 2004. Effects of forest edges, fruit display size, and fruit colour on bird seed dispersal in a New Zealand mistletoe, *Alepis flavida*. *New Zealand Journal of Ecology* **28**:93-103.
- Barker, F. K., A. Cibois, P. Schikler, J. Feinstein, and J. Cracraft. 2004. Phylogeny and diversification of the largest avian radiation. *Proceedings of the National Academy of Sciences of the United States of America* **101**:11040-11045.
- Barlow, B. A. 1983. Biogeography of Loranthaceae and Viscaceae. Pages 19-46 in D. M. Calder and P. Bernhardt, editors. *The Biology of Mistletoes*. Academic Press, Sydney.
- Barlow, B. A. and D. Weins. 1971. The cytogeography of the Loranthaceous mistletoes. *Taxon* **20**:291-312.
- Basabose, A. K. 2002. Diet composition of chimpanzees inhabiting the montane forest of Kahuzi, Democratic Republic of Congo. *American Journal of Primatology* **58**:1-21.

- Bascompte, J. and P. Jordano. 2007. Plant-animal mutualistic networks: The architecture of biodiversity. Pages 567-593. *Annual Review of Ecology, Evolution, and Systematics*.
- Bawa, K. S. 1990. Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics* **21**:399-422.
- Beck, J. and H. Chapman. 2008. A population estimate of the endangered chimpanzee *Pan troglodytes vellerosus* in a Nigerian montane forest: implications for conservation. *Oryx* **42**:448-451.
- Beeson, M., S. Tame, E. Keeming, and S. E. G. Lea. 1996. Food habits of guenons (*Cercopithecus* spp.) in Afro-montane forest. *African Journal of Ecology* **34**:202-210.
- Begho, E. R., K. O. Omokhafa, E. E. Omo-Ikerodah, and E. O. Akpaja. 2001. Some Observations on the Fruit Set and Incidence of Mistletoes on Rubber Trees in Nigeria. *American-Eurasian Journal of Sustainable Agriculture* **1**:13-18.
- Bernhardt, P. 1983. The floral biology of *Amyema* in South-Eastern Australia. Pages 87-100 in D. M. Calder and P. Bernhardt, editors. *The Biology of Mistletoes*. Academic Press, Australia.
- Bernhardt, P. 1984. Mistletoes on mistletoes: the floral ecology of *Amyema miraculosum* and its host, *Amyema miquelii* (Loranthaceae). *Australian Journal of Botany* **32**:73-86.
- Bernhardt, P., and D. M. Calder. 1981. The floral ecology of sympatric populations of *Amyema pendulum* and *Amyema quandang* (Loranthaceae). *Bulletin of the Torrey Botanical Club* **108**:213-230.
- Bernhardt, P., R. B. Knox, and D. M. Calder. 1980. Floral Biology and Self-Incompatibility in Some Australian Mistletoes of the Genus *Amyema* (Loranthaceae). *Australian Journal of Botany* **28**:437-451.
- Bhatnagar, S. P. and B. M. Johri. 1983. Embryology of Loranthaceae. Pages 47-67 in D. M. Calder and P. Bernhardt, editors. *The Biology of Mistletoes*. Academic Press, Sydney.
- Bond, W. J. 1994. Do Mutualisms Matter? Assessing the Impact of Pollinator and Disperser Disruption on Plant Extinction. *Philosophical Transactions: Biological Sciences* **344**:83-90.
- Borrow, N. and R. Demey. 2001. *A guide to the birds of Western Africa*. Princeton University Press, Oxford, UK.
- Bright, E. O., S. M. Misari, and S. Ogunyemi. 2001. Preliminary study on the host range and dispersal of mistletoe (*Tapinanthus globiferus*). *Nigerian Journal of Weed Science* **14**:53-58.
- Burgess, N. D., A. Balmford, N. J. Cordeiro, J. Fjeldsa, W. Kuper, C. Rahbek, E. W. Sanderson, J. P. W. Scharlemann, J. H. Sommer, and P. H. Williams. 2007. Correlations among species distributions, human density and human infrastructure across the high biodiversity tropical mountains of Africa. *Biological Conservation* **134**:164-177.

- Burgess, V. J., D. Kelly, A. W. Robertson, and J. J. Ladley. 2006. Positive effects of forest edges on plant reproduction: Literature review and a case study of bee visitation to flowers of *Peraxilla tetrapetala* (Loranthaceae). *New Zealand Journal of Ecology* **30**:179-190.
- Calder, D. M. 1983. Mistletoes in focus: an introduction. Pages 1-18 in D. M. Calder and P. Bernhardt, editors. *The Biology of Mistletoes*. Academic Press, Sydney.
- Calvin, C. and C. Wilson. 1998. Comparative morphology of haustoria within African Loranthaceae. Pages 17-36 in R. M. Polhill and D. Weins, editors. *The Mistletoes of Africa*. Royal Botanic Gardens Kew, Richmond-Surrey, UK.
- Campbell, D. R. 1985. Pollinator sharing and seed set of *Stellaria pubera*: Competition for pollination. *Ecology* **66**:544-553.
- Carlo, T. A. 2005. Interspecific neighbors change seed dispersal pattern of an avian-dispersed plant. *Ecology* **86**:2440-2449.
- Carlo, T. A. and J. M. Morales. 2008. Inequalities in fruit-removal and seed dispersal: Consequences of bird behaviour, neighbourhood density and landscape aggregation. *Journal of Ecology* **96**:609-618.
- Chalcoff, V. R., M. A. Aizen, and L. Galetto. 2006. Nectar concentration and composition of 26 species from the temperate forest of South America. *Annals of Botany* **97**:413-421.
- Chapman, C. A. and L. J. Chapman. 1996. Frugivory and the fate of dispersed and non-dispersed seeds of six African tree species. *Journal of Tropical Ecology* **12**:491-504.
- Chapman, H. M. and J. D. Chapman. 2001. *The Forest Flora of Taraba and Adamawa States, Nigeria: An ecological account and plant species checklist*. University of Canterbury, Christchurch.
- Chapman, H. M., S. M. Olson, and D. Trumm. 2004. An assessment of changes in the montane forests of Taraba State, Nigeria, over the past 30 years. *Oryx* **38**:282-290.
- Charlesworth, D. and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual review of ecology and systematics*. Vol. 18:237-268.
- Cheek, M., J. Onana, and B. Pollard. 2000. *The Plants of Mount Oku and the Ijim Ridge, Cameroon: A conservation checklist*. Royal Botanic Gardens, Kew, UK.
- Cheke, R. A. and C. F. Mann. 2001. *Sunbirds: a guide to the sunbirds, flowerpeckers, spiderhunters, and sugarbirds of the world*. Yale University Press, New Haven, CT.
- Cipollini, M. L. and D. J. Levey. 1997. Secondary metabolites of fleshy vertebrate-dispersed fruits: Adaptive hypotheses and implications for seed dispersal. *American Naturalist* **150**:346-372.
- Cordeiro, N. J. and H. F. Howe. 2001. Low recruitment of trees dispersed by animals in African forest fragments. *Conservation Biology* **15**:1733-1741.

- Cordeiro, N. J. and H. F. Howe. 2003. Forest Fragmentation Severs Mutualism between Seed Dispersers and an Endemic African Tree. *Proceedings of the National Academy of Sciences of the United States of America* **100**:14052-14056.
- Corlett, R. T. 2007. Pollination or seed dispersal: which should we worry about the most? Pages 523-544 in A. J. Dennis, E. W. Schupp, R. J. Green, and D. A. Westcott, editors. *Seed dispersal: theory and its application in a changing world*. CAB International, Wallingford, UK.
- Cox, P. A. 1991. Abiotic pollination: an evolutionary escape for animal-pollinated angiosperms. *Philosophical Transactions - Royal Society of London, B* **333**:217-224.
- Cruden, R. W. 1972. Pollinators in High-Elevation Ecosystems: Relative Effectiveness of Birds and Bees. *Science* **176**:1439-1440.
- Culley, T. M., S. G. Weller, and A. K. Sakai. 2002. The evolution of wind pollination in angiosperms. *Trends in Ecology and Evolution* **17**:361-369.
- D'Arcy, W. G. 1985. Gorillas and mistletoes in Central Africa. *The Golden Bough* **6**:1-2.
- Dale, S., K. Mork, R. Solvang, and A. J. Plumptre. 2000. Edge effects on the understory bird community in a logged forest in Uganda. *Conservation Biology* **14**:265-276.
- Danser, B. H. 1933. A new system for the genera of Loranthaceae Loranthoideae, with a nomenclator for the Old World species of this subfamily. *Verhhandelingen der Koninklijke Akademie van Wetenschappen te Amsterdam Afdeeling Natuurkunde, sect. 2*. **29**:1-128.
- Darwin, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. http://darwin-online.org.uk/pdf/1859_Origin_F373.pdf. John Murray, London.
- Davidar, P. 1983a. Birds and neotropical mistletoes: effects on seedling recruitment. *Oecologia* **60**:271-273.
- Davidar, P. 1983b. Similarity between flowers and fruits in some flowerpecker pollinated mistletoes. *Biotropica* **15**:32-37.
- Davidar, P. 1984. Ecological interactions between mistletoes and their avian pollinators in South India. *Journal of the Bombay Natural History Society* **82**:45-60.
- Davidar, P. and E. S. Morton. 1986. The relationship between fruit crop sizes and fruit removal rates by birds. *Ecology* **67**:262-265.
- Delph, L. F. and C. M. Lively. 1989. The evolution of floral color change: pollinator attraction versus physiological constraints in *Fuchsia excorticata*. *Evolution* **43**:1252-1262.
- Der, J. P. and D. L. Nickrent. 2008. A molecular phylogeny of Santalaceae (Santalales). *Systematic Botany* **33**:107-116.

- Devkota, M. P. and G. Glatzel. 2005. Mistletoes of the Annapurna Conservation Area, Central Nepal Himalayas. *Journal of Japanese Botany* **80**:27-36.
- Devy, M. S. and P. Davidar. 2006. Breeding systems and pollination modes of understorey shrubs in a medium elevation wet evergreen forest, southern Western Ghats, India. *Current Science* **90**:838-842.
- Docters Van Leeuwen, W. M. 1954. On the biology of some Javanese Loranthaceae and the role birds play in their life-history. *Beaufortia* **4**:105-205.
- Dowsett-Lemaire, F. 1982. Tinkerbirds and mistletoes on the Nyika Plateau, south-central Africa. *The Golden Bough* **1**:3.
- Dowsett-Lemaire, F. 1985. The forest vegetation of the Nyika Plateau (Malawi-Zambia): Ecological and phenological studies. *Bull. Jard. Bot. Nat. Belg.* **55**:301-392.
- Dowsett-Lemaire, F. 1988. Fruit choice and seed dissemination by birds and mammals in the evergreen forests of upland Malawi. *Revue d'Ecologie (Terre et Vie)* **43**:251-285.
- Dowsett-Lemaire, F. 1989. Food plants and the annual cycle in a montane community of sunbirds (*Nectarinia* spp.) in northern Malawi. *Tauraco* **1**:167-185.
- Engler, A. 1889. Die natürlichen Pflanzenfamilien III in A. Engler and K. Prantl, editors. Loranthaceae. W. Engelmann, Leipzig.
- Evans, M. S. 1895. The fertilisation of *Loranthus kraussianus* and *L. dregei*. *Nature* **51**:235-236.
- Farwig, N., K. Bohning-Gaese, and B. Bleher. 2006. Enhanced seed dispersal of *Prunus africana* in fragmented and disturbed forests? *Oecologia* **147**:238-252.
- Feehan, J. 1985. Explosive flower opening in ornithophily: a study of pollination mechanisms in some Central African Loranthaceae. *Botanical Journal of the Linnean Society* **90**:129-144.
- Feinsinger, P., H. M. Tiebout III, and B. E. Young. 1991. Do tropical bird-pollinated plants exhibit density-dependent interactions? Field experiments. *Ecology* **72**:1953-1963.
- Fleming, T. H. and N. Muchhala. 2008. Nectar-feeding bird and bat niches in two worlds: Pantropical comparisons of vertebrate pollination systems. *Journal of Biogeography* **35**:764-780.
- Ford, H. A. 1979. Interspecific competition in Australian honeyeaters - depletion of common resources. *Australian Journal of Ecology* **4**:145 - 164.
- Fred-Jaiyesimi, A., T. Onabanjo, and O. Ademuyiwa. 2008. Phytochemical and laxative studies of *Globimetula braunii* (Engler) van Tieghem growing on *Cola Acuminata* (Schott & Endl) African Journal of Traditional, Complementary and Alternative Medicines **5**:419-420.

- French, K. and M. Westoby. 1992. Removal of vertebrate-dispersed fruits in vegetation on fertile and infertile soils. *Oecologia* **91**:447-454.
- Frost, S. K. and P. G. H. Frost. 1981. Sunbird pollination of *Strelitzia nicolai*. *Oecologia* **49**:379-384.
- Galetti, M., C. P. Alves-Costa, and E. Cazetta. 2003. Effects of forest fragmentation, anthropogenic edges and fruit colour on the consumption of ornithocoric fruits. *Biological Conservation* **111**:269-273.
- Galetto, L. and G. Bernardello. 2005. Nectar. Pages 261-313 in A. Dafni, P. G. Kevan, and B. C. Husband, editors. *Practical Pollination Biology*. Enviroquest Ltd., Cambridge, Ontario.
- Geerts, S. and A. Pauw. 2009. African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. *Oikos* **118**:573-579.
- Gervais, J. A., B. R. Noon, and M. F. Willson. 1999. Avian selection of the color-dimorphic fruits of salmonberry, *Rubus spectabilis*: A field experiment. *Oikos* **84**:77.
- Giles, S. and A. Lill. 1999. The effect of fruit abundance, conspicuousness and sugar concentration on fruit colour choice by captive silvereyes. *Ethology Ecology and Evolution* **11**:229.
- Gill, F. B. and L. L. Wolf. 1975. Foraging Strategies and Energetics of East African Sunbirds at Mistletoe Flowers. *The American Naturalist* **109**:491-510.
- Gill, F. B. and L. L. Wolf. 1978. Comparative Foraging Efficiencies of Some Montane Sunbirds in Kenya *The Condor* **80**:391-400
- Gill, F. B. and L. L. Wolf. 1979. Non-random foraging by sunbirds in a patchy environment. *Ecology* **58**:1284-1296.
- Githiru, M., L. Lens, L. A. Bennur, and C. P. K. O. Ogol. 2002. Effects of site and fruit size on the composition of avian frugivore assemblages in a fragmented Afrotropical forest. *Oikos* **96**:320-330.
- Godschalk, S. 1983a. The morphology of some South African mistletoe fruits. *South African Journal of Botany* **2**:52-56.
- Godschalk, S. K. B. 1983b. Mistletoe Dispersal by Birds in South Africa. Pages 117-128 *The Biology of Mistletoes*. Academic Press, Sydney.
- Godschalk, S. K. B. 1985. Feeding behaviour of avian dispersers of mistletoe fruit in the Loskop Dam Nature Reserve, South Africa. *South African Journal of Zoology* **20**:136-146.
- Goodwillie, C., S. Kalisz, and C. G. Eckert. 2005. The evolutionary enigma of mixed mating systems in plants: Occurrence, Theoretical Explanations, and Empirical Evidence. *Annual Review of Ecology, Evolution, and Systematics* **36**:47-79.

- Gori, D. F. 1983. Post-pollination phenomena and adaptive floral changes. *in* C. E. Jones and R. J. Little, editors. *Handbook of Experimental Pollination Biology*. Van Nostrand Rhinehold, New York.
- Goulson, D., J. L. Cruise, K. R. Sparrow, A. J. Harris, K. J. Park, M. C. Tinsley, and A. S. Gilburn. 2007. Choosing rewarding flowers; perceptual limitations and innate preferences influence decision making in bumblebees and honeybees. *Behavioral Ecology and Sociobiology* **61**:1523-1529.
- Graham, C. H., T. C. Moermond, K. A. Kristensen, and J. Mvukiyumwami. 1995. Seed Dispersal Effectiveness by Two Bulbuls on *Maesa lanceolata*, an African Montane Forest Tree. *Biotropica* **27**:479-486.
- Grant, V. 1950. The Protection of the Ovules in Flowering Plants. *Evolution* **4**:179-201.
- Green, A. K., D. Ward, and M. E. Griffiths. 2008. Directed dispersal of mistletoe (*Plicosepalus acaciae*) by Yellow-vented Bulbuls (*Pycnonotus xanthopygos*). *Journal of Ornithology*:1-7.
- Grimshaw, J. M. 2001. What do we really know about the Afrotropical Archipelago? *Systematics and Geography of Plants* **71**:949-957.
- Gross-Camp, N. and B. A. Kaplin. 2005. Chimpanzee (*Pan troglodytes*) seed dispersal in an afrotropical forest: Microhabitat influences on the postdispersal fate of large seeds. *Biotropica* **37**:641-649.
- Gross, C. L. 1996. Is resource overlap disadvantageous to three sympatric legumes? *Australian Journal of Ecology* **21**:133-143.
- Gross, R. S. and P. A. Werner. 1983. Relationships among flowering phenology, insect visitors, and seed-set of individuals: Experimental studies on four co-occurring species of goldenrod (*Solidago*: Compositae). *Ecological Monographs* **53**:95-117.
- Hargreaves, A. L., L. D. Harder, and S. D. Johnson. 2009. Consumptive emasculation: The ecological and evolutionary consequences of pollen theft. *Biological Reviews* **84**:259-276.
- Herrera, C. M. 1984. A study of avian frugivores, bird-dispersed plants, and their interaction in Mediterranean scrublands. *Ecological Monographs* **54**:1-23.
- Herrera, C. M. 1995. Plant-vertebrate seed dispersal systems in the Mediterranean: ecological, evolutionary and historical determinants. *Annual Review of Ecology and Systematics* **26**:705-727.
- Herrera, C. M. 2002. Seed dispersal by vertebrates. Pages 185 -208 *in* C. M. Herrera and O. Pellmyr, editors. *Plant-Animal Interactions: An Evolutionary Approach*. Blackwell Science, Oxford, UK.

- Hoffmann, A. J., E. R. Fuentes, I. Cortes, F. Liberona, and V. Costa. 1986. *Tristerix tetrandrus* (Loranthaceae) and its host-plants in the Chilean matorral: patterns and mechanisms. *Oecologia* **69**:202-206.
- Honkavaara, J., H. Siitari, and J. Viitala. 2004. Fruit colour preferences of redwings (*Turdus iliacus*): Experiments with hand-raised juveniles and wild-caught adults. *Ethology* **110**:445.
- Howe, H. F. and G. F. Estabrook. 1977. On intraspecific competition for avian dispersers in tropical trees. *American Naturalist* **111**:817-832.
- Howe, H. F. and J. Smallwood. 1982. Ecology of Seed Dispersal. *Annual Review of Ecology and Systematics* **13**:201-228.
- Howe, H. F. and L. C. Westley. 1986. Ecology of pollination and seed dispersal. Pages 185 - 215 in M. J. Crawley, editor. *Plant Ecology*. Blackwells Scientific, Oxford.
- Ie, O. and N. Zam. 2008. Oxytotic properties of the aqueous extract of *Globimetula braunii* (loranthaceae). *Pakistan journal of pharmaceutical sciences* **21**:356-360.
- Ihuma, J. O. 2006. The Influence of frugivore communities on forest fragment regeneration in Ngel Nyaki Forest Reserve, Taraba State, Nigeria
MSc. Federal University of Technology, Yola.
- Inouye, D. W. 1980. The terminology of floral larceny. *Ecology* **61**:1251-1253.
- Irwin, M. P. S. 1999. The genus *Nectarinia* and the evolution and diversification of sunbirds: An Afrotropical perspective. *Honeyguide* **45**:45-58.
- Izhaki, I. 2002. The role of fruit traits in determining fruit removal in east mediterranean ecosystems. Pages 161-175 in D. J. Levey, A. Silva, and M. Galetti, editors. *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation*. CABI, New York.
- Izhaki, I., E. Tsahar, I. Paluy, and J. Friedman. 2002. Within population variation and interrelationships between morphology, nutritional content, and secondary compounds of *Rhamnus alaternus* fruits. *New Phytologist* **156**:217-223.
- Janzen, D. H. 1980. When is it Coevolution? *Evolution* **34**:611-612.
- Johnson, S. D. 1996. Bird pollination in South African species of *Satyrium* (Orchidaceae). *Plant Systematics & Evolution* **203**:91-98.
- Johnson, S. D. and M. Brown. 2004. Transfer of pollinaria on birds' feet: a new pollination system in orchids. *Plant Systematics & Evolution* **244**:181-188.
- Johnson, S. D. and K. E. Steiner. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution* **15**:140-143.
- Johow, F. 1900. Zur Bestubungsbiologie chilenischer Bluthen. I. Verhandl. Deutsch. Wiss. Ver. Santiago **4**.

- Jordano, P. 1987a. Avian fruit removal: effects of fruit variation, crop size, and insect damage. *Ecology* **68**:1711-1723.
- Jordano, P. 1987b. Patterns of Mutualistic Interactions in Pollination and Seed Dispersal: Connectance, Dependence Asymmetries, and Coevolution. *The American Naturalist* **129**:657-677.
- Jordano, P. 1989. Pre-dispersal biology of *Pistacia lentiscus* (Anacardiaceae): cumulative effects on seed removal by birds. *Oikos* **55**:375-386.
- Jordano, P. 1992. Fruits and Frugivory. Pages 105-156 in M. Fenner, editor. *Seeds: The Ecology of Regeneration in Plant Communities*. C.A.B International, Wallingford, UK.
- Jordano, P. 1995a. Angiosperm fleshy fruits and seed dispersers: A comparative analysis of adaptation and constraints in plant-animal interactions. *American Naturalist* **145**:163-191.
- Jordano, P. 1995b. Frugivore-mediated selection on fruit and seed size: Birds and St.Lucie's cherry, *Prunus mahaleb*. *Ecology* **76**:2627-2639.
- Jordano, P. and E. W. Schupp. 2000. Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs* **70**:591-615.
- Kaplin, B. A. and T. C. Moermond. 1998. Variation in seed handling by two species of forest monkeys in Rwanda. *American Journal of Primatology* **45**:83-101.
- Kaplin, B. A. and T. C. Moermond. 2000. Foraging ecology of the mountain monkey (*Cercopithecus lhoesti*): Implications for its evolutionary history and use of disturbed forest. *American Journal of Primatology* **50**:227-246.
- Kaplin, B. A., V. Munyaligoga, and T. C. Moermond. 1998. The influence of temporal changes in fruit availability on diet composition and seed handling in blue monkeys (*Cercopithecus mitis doggetti*). *Biotropica* **30**:56-71.
- Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* **29**:83-112.
- Keay, R. W. J. 1958. *Flora of West Tropical Africa*. 1(2), 2nd edition. The Crown agents for Oversea Governments and Administrations, London.
- Kelly, D., J. J. Ladley, A. Robertson, and D. A. Norton. 2000. Limited forest fragmentation improves reproduction in the declining New Zealand mistletoe *Peraxilla tetrapetala* (Loranthaceae). Pages 241-252 in A. G. Young and G. Clarke, editors. *Genetics, demography and viability of fragmented populations*. Cambridge University Press, Cambridge.
- Kelly, D., J. J. Ladley, and A. W. Robertson. 2004. Is dispersal easier than pollination? Two tests in New Zealand Loranthaceae. *New Zealand Journal of Botany* **42**:89-103.
- Kelly, D., J. J. Ladley, and A. W. Robertson. 2007. Is the pollen-limited mistletoe *Peraxilla tetrapetala* (Loranthaceae) also seed limited? *Austral Ecology* **32**:850-857.

- Kelly, D., J. J. Ladley, A. W. Robertson, and L. Crowfoot. 2008. Flower predation by *Zelleria maculata* (Lepidoptera) on *Peraxilla* mistletoes: Effects of latitude and fragmentation, and impact on fruit set. *New Zealand Journal of Ecology* **32**:186.
- Kelly, D., J. J. Ladley, A. W. Robertson, J. Edwards, and D. C. Smith. 1996. The birds and the bees. *Nature* **384**:615.
- Kirika, J. M., N. Farwig, and K. Bohning-Gaese. 2008. Effects of local disturbance of tropical forests on frugivores and seed removal of a small-seeded Afrotropical tree. *Conservation Biology* **22**:318-328.
- Kirkup, D. W. 1984. Avian visitors to some West African mistletoes. *Golden Bough* **5**:3-4.
- Kirkup, D. W. 1993. The structural basis of pollination in African Loranthaceae. Ph.D. Thesis. University of Reading.
- Kirkup, D. W. 1998. Pollination Mechanisms in African Loranthaceae. Pages 37-60 in R. M. Polhill and D. Weins, editors. *Mistletoes of Africa*. Royal Botanic Gardens, Kew, London.
- Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005. Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology Evolution and Systematics* **36**:467-497.
- Kuijt, J. 1968. Mutual affinities of Santalalean families. *Brittonia* **20**:136-147.
- Kuijt, J. 1988. Revision of *Tristerix* (Loranthaceae). *Systematic Botany Monographs* **19**:1-61.
- Kujit, J. 1969. *The Biology of Parasitic Flowering Plants*. University of California Press, Los Angeles.
- Kwit, C., D. J. Levey, C. H. Greenberg, S. F. Pearson, J. P. McCarty, and S. Sargent. 2004. Cold temperature increases winter fruit removal rate of a bird-dispersed shrub. *Oecologia* **139**:30-34.
- Ladley, J. J. 1994. The Reproductive Ecology of the Loranthaceae Mistletoes of New Zealand. M.Sc. thesis. University of Canterbury.
- Ladley, J. J. and D. Kelly. 1995. Explosive New Zealand mistletoe *Nature* **378**:766.
- Ladley, J. J. and D. Kelly. 1996. Dispersal, germination and survival of New Zealand mistletoes (Loranthaceae): Dependence on birds. *New Zealand Journal of Ecology* **20**:69-79.
- Ladley, J. J., D. Kelly, and A. W. Robertson. 1997. Explosive flowering, nectar production, breeding systems, and pollinators of New Zealand mistletoes (Loranthaceae). *New Zealand Journal of Botany* **35**:345-360.
- Lambert, J. E. 1999. Seed handling in chimpanzees (*Pan troglodytes*) and redbell monkeys (*Cercopithecus ascanius*): Implications for understanding hominoid and cercopithecine

- fruit-processing strategies and seed dispersal. *American Journal of Physical Anthropology* **109**:365-386.
- Lambert, J. E. 2002. Exploring the link between animal frugivory and plant strategies: The case of primate fruit processing and post-dispersal seed fate. *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation*:365-379.
- Lamont, B. 1983. Germination of mistletoes. Pages 129-143 *in* D. M. Calder and P. Bernhardt, editors. *The Biology of Mistletoes*. Academic Press, Sydney.
- Lamont, B. 1985. Dispersal of the winged fruits of *Nuytsia floribunda* (Loranthaceae). *Australian Journal of Ecology* **10**:187-193.
- Lamont, B. and M. Perry. 1977. The effects of light, osmotic potential and atmospheric gases on germination of the mistletoe *Amyema preissii*. *Annals of Botany* **41**:203-209.
- Larson, B. M. H. and S. C. H. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* **69**:503-520.
- Lemon, W. C. 1991. Fitness consequences of foraging behaviour in the zebra finch. *Nature* **352**:153-155.
- Levey, D. J., T. C. Moermond, and J. S. Denslow. 1984. Fruit choice in neotropical birds: the effect of distance between fruits on preference patterns. *Ecology* **65**:844-850.
- Liddy, J. 1983. Dispersal of Australian Mistletoes: Cowiebank Study. Pages 101-116 *in* D. M. Calder and P. Bernhardt, editors. *The Biology of Mistletoes*. Academic Press, Sydney.
- Lopez de Buen, L. and J. F. Ornelas. 1999. Frugivorous birds, host selection and the mistletoe *Psittacanthus schiedeana*, in central Veracruz, Mexico. *Journal of Tropical Ecology* **15**:329-340.
- Lopez De Buen, L. and J. F. Ornelas. 2001. Seed dispersal of the mistletoe *Psittacanthus schiedeana* by Birds in Central Veracruz, Mexico. *Biotropica* **33**:487-494.
- Lyaruu, H. V., S. Eliapenda, and I. Backeus. 2000. Floristic, structural and seed bank diversity of a dry Afromontane forest at Mafai, central Tanzania. *Biodiversity and Conservation* **9**:241-263.
- Maisels, F., E. Kemei, M. Kemei, and C. Toh. 2001. The extirpation of large mammals and implications for montane forest conservation: the case of the Kilum-Ijim Forest, North-west Province, Cameroon. *Oryx* **35**:322-331.
- Maisels, F. G. and P. F. Forboseh. 1999. Phenology of the major tree and shrub species of Kilum-Ijim forest. Birdlife International/MINEF, Cameroon.
- Malecot, V. and D. L. Nickrent. 2008. Molecular phylogenetic relationships of Olacaceae and related Santalales. *Systematic Botany* **33**:97-106.
- Maloof, J. E. and D. W. Inouye. 2000. Are nectar robbers cheaters or mutualists? *Ecology* **81**:2651-2661.

- Manasse, R. S. and H. F. Howe. 1983. Competition for dispersal agents among tropical trees: influences of neighbors. *Oecologia* **59**:185-190.
- Martinez del Rio, C., M. Hourdequin, A. Silva, and R. Medel. 1995. The influence of cactus size and previous infection on bird deposition of mistletoe seeds. *Australian Journal of Ecology* **20**:571-576.
- Martinez del Rio, C., A. Silva, R. Medel, and M. Hourdequin. 1996. Seed Dispersers as Disease Vectors: Bird Transmission of Mistletoe Seeds to Plant Hosts. *Ecology* **77**:912-921.
- Masaki, T., Y. Kominami, and T. Nakashizuka. 1994. Spatial and seasonal patterns of seed dissemination of *Cornus controversa* in a temperate forest. *Ecology* **75**:1903-1910.
- McCarty, J. P., D. J. Levey, C. H. Greenberg, and S. Sargent. 2002. Spatial and temporal variation in fruit use by wildlife in a forested landscape. *Forest Ecology and Management* **164**:277-291.
- Medan, D., N. H. Montaldo, M. Devoto, A. Mantese, V. Vasellati, G. G. Roitman, and N. H. Bartoloni. 2002. Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. *Arctic, Antarctic, and Alpine Research* **34**:233-241.
- Medel, R., E. Vergara, A. Silva, and M. Kalin-Arroyo. 2004. Effects of vector behavior and host resistance on mistletoe aggregation. *Ecology* **85**:120-126.
- Memmott, J. 1999. The structure of a plant-pollinator food web. *Ecology Letters* **2**:276-280.
- Menzies, B. P. 1947. Some Observations on the Parasitic Species *Loranthus Micranthus* (Hook). Unpublished M.Sc. thesis. University of Auckland, New Zealand.
- Monteiro, R. F., R. P. Martins, and K. Yamamoto. 1992. Host specificity and seed dispersal of *Psittacanthus robustus* (Loranthaceae) in South-East Brazil. *Journal of Tropical Ecology* **8**:307-314.
- Murcia, C. 1995. Edge effects in fragmented forests: Implications for conservation. *Trends in Ecology and Evolution* **10**:58-62.
- Murphy, D. J. and D. Kelly. 2001. Scarce or distracted? Bellbird (*Anthornis melanura*) foraging and diet in an area inadequate mistletoe pollination. *New Zealand Journal of Ecology* **25**:69-81.
- Murray, K. G. 1987. Selection for optimal fruit-crop size in bird-dispersed plants. *American Naturalist* **129**:18-31.
- Norton, D. A., R. J. Hobbs, and L. Atkins. 1995. Fragmentation, disturbance, and plant distribution: Mistletoes in woodland remnants in the Western Australian wheatbelt. *Conservation Biology* **9**:426-438.
- Norton, D. A. and J. J. Ladley. 1998. Establishment and early growth of *Alepis flavida* in relation to *Nothofagus solandri* branch size. *New Zealand Journal of Botany* **36**:213-217.

- Norton, D. A., J. J. Ladley, and H. J. Owen. 1997. Distribution and population structure of the loranthaceous mistletoes *Alepis flavida*, *Peraxilla colensoi*, and *Peraxilla tetrapetala* within two New Zealand *Nothofagus* forests. *New Zealand Journal of Botany* **35**:323-336.
- Norton, D. A. and N. Reid. 1997. Lessons in ecosystem management from management of threatened and pest Loranthaceous mistletoes in New Zealand and Australia. *Conservation Biology* **11**.
- O'Donnell, C. F. and P. J. Dilks. 1994. Foods and foraging of forest birds in temperate rainforest, South Westland, New Zealand. *New Zealand Journal of Ecology* **18**:87-107.
- Obeso, J. R. and C. M. Herrera. 1994. Inter- and intraspecific variation in fruit traits in co-occurring vertebrate-dispersed plants. *International Journal of Plant Sciences* **155**:382-387.
- Olagunju, J. A., F. I. Ismail, and Z. O. Gbile. 1999. The hypoglycaemic property of normal saline leaf extract of *Globimetula braunii* in alloxanized diabetic albino rats. *Biomedical Letters* **60**:83-89.
- Ollerton, J. 1998. Sunbird surprise for syndromes. *Nature* **394**:726-727.
- Paine, R. T. 1969. A note on trophic complexity and community stability. *Am. Nat.* **103**:91-93.
- Parker, T. A. 1981. Distribution and biology of the White-cheeked Continga *Zaratornis stresemanni*, a high Andean frugivore. *Bulletin of the British Ornithological Club* **101**(1):256-265.
- Paton, D. C. and H. A. Ford. 1977. Pollination by birds of native plants in South Australia. *Emu* **77**:73-85.
- Pauw, A. 1998. Pollen transfer on birds' tongues. *Nature* **394**:731-732.
- Peck, J. R., J. M. Yearsley, and D. Waxman. 1998. Explaining the geographic distributions of sexual and asexual populations. *Nature* **391**:889-892.
- Polhill, R. M. 1989. Speciation patterns in African Loranthaceae. Pages 221-236 in L. B. Holm-Nielsen, I. C. Nielsen, and H. Balslev, editors. *Tropical forests : botanical dynamics, speciation and diversity* Academic Press, London.
- Polhill, R. M. and D. Wiens. 1998. *Mistletoes of Africa*. Royal Botanic Gardens Kew, London.
- Powell, J. A. 1992. Interrelationships of yuccas and yucca moths. *Trends in Ecology and Evolution* **7**:10-15.
- Primack, R. B. 1985. Longevity of individual flowers. *Annual review of ecology and systematics*. Vol. 16:15-37.
- Proctor, M., P. Yeo, and A. Lack. 1996. *The Natural History of Pollination*. Timber Press, Portland, Oregon.

- Puckey, H. L., A. Lill, and D. J. O'Dowd. 1996. Fruit color choices of captive Silvereyes (*Zosterops lateralis*). *Condor* **98**:780.
- Pyke, G. H. 1984. Optimal foraging theory: a critical review. *Annual review of ecology and systematics*. Vol. 15:523-575.
- Raven, P. H. 1972. Why are Bird-Visited Flowers Predominantly Red? *Evolution* **26**:674.
- Regal, P. J. 1982. Pollination by wind and animals: ecology of geographic patterns. *Annual review of ecology and systematics*. Volume 13:497-524.
- Reid, N. 1986. Pollination and seed dispersal of mistletoes (Loranthaceae) by birds in Southern Australia. Pages 64-84 in H. A. Ford and D. C. Paton, editors. *The dynamic partnership. Birds and plants in Southern Australia*. Government Printer, Adelaide.
- Reid, N. 1987. The mistletoebird and Australian mistletoes: Coevolution or coincidence? *Emu* **87**:130-131.
- Reid, N. 1989. Dispersal of mistletoes by honeyeaters and flowerpeckers: components of seed dispersal quality. *Ecology* **70**:137-145.
- Reid, N. 1990. Mutualistic interdependence between mistletoes (*Amyema quandang*), and spiny-cheeked honeyeaters and mistletoebirds in an arid woodland. *Australian Journal of Ecology* **15**:175-190.
- Reid, N. 1991. Coevolution of mistletoes and frugivorous birds? *Australian Journal of Ecology* **16**:457-469.
- Restrepo, C., N. Gomez, and S. Heredia. 1999. Anthropogenic edges, treefall gaps, and fruit-frugivore interactions in a neotropical montane forest. *Ecology* **80**:668-685.
- Restrepo, C., S. Sargent, D. J. Levey, and D. M. Watson. 2002. The Role of Vertebrates in the Diversification of New World Mistletoes. Pages 83-98 in D. J. Levey, W. R. Silva, and M. Galetti, editors. *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation*. CAB International New York, USA.
- Richards, A. J. 1996. Breeding systems in flowering plants and the control of variability. *Folia Geobotanica* **31**:283-293.
- Richardson, K. C. and R. D. Wooller. 1988. The alimentary tract of a specialist frugivore, the mistletoebird, *Dicaeum hirundinaceum*, in relation to its diet. *Aust. J. Zool.* **36**:373-382.
- Rivera, G. L., L. Galetto, and L. Bernardello. 1996. Nectar secretion pattern, removal effects, and breeding system of *Ligaria cuneifolia* (Loranthaceae). *Canadian Journal of Botany* **74**:1996-2001.
- Robertson, A. W., D. Kelly, J. J. Ladley, and A. D. Sparrow. 1999. Effects of pollinator loss on endemic New Zealand mistletoes (Loranthaceae). *Conservation Biology* **13**:499-508.

- Robertson, A. W., J. J. Ladley, and D. Kelly. 2005. Effectiveness of short-tongued bees as pollinators of apparently ornithophilous New Zealand mistletoes. *Austral Ecology* **30**:298-309.
- Robertson, A. W., J. J. Ladley, and D. Kelly. 2008. Does height off the ground affect bird visitation and fruit set in the pollen-limited mistletoe *Peraxilla tetrapetala* (Loranthaceae)? *Biotropica* **40**:122-126.
- Robertson, A. W., A. Trass, J. J. Ladley, and D. Kelly. 2006. Assessing the benefits of frugivory for seed germination: The importance of the deinhibition effect. *Functional Ecology* **20**:58-66.
- Rodger, J. G., K. Balkwill, and B. Gemmill. 2004. African pollination studies: where are the gaps? *International Journal of Tropical Insect Science* **24**:5-28.
- Rodriguez-Cabal, M. A., M. A. Aizen, and A. J. Novaro. 2007. Habitat fragmentation disrupts a plant-disperser mutualism in the temperate forest of South America. *Biological Conservation* **139**:195-202.
- Roxburgh, L. 2007. The effect of gut processing on the quality of mistletoe seed dispersal. *Journal of Tropical Ecology* **23**:377-380.
- Sallabanks, R. 1992. Fruit fate, frugivory, and fruit characteristics: a study of the hawthorn, *Crataegus monogyna* (Rosaceae). *Oecologia* **91**:296-304.
- Sampson, B. J., R. G. Danka, and S. J. Stringer. 2004. Nectar robbery by bees *Xylocopa virginica* and *Apis mellifera* contributes to the pollination of rabbiteye blueberry. *Journal of Economic Entomology* **97**:735-740.
- Samuels, I. A. and D. J. Levey. 2005. Effects of gut passage on seed germination: Do experiments answer the questions they ask? *Functional Ecology* **19**:365-368.
- Saracco, J. F., J. A. Collazo, M. J. Groom, and T. A. Carlo. 2005. Crop size and fruit neighborhood effects on bird visitation to fruiting *Schefflera morototoni* trees in Puerto Rico. *Biotropica* **37**:81-87.
- Sargent, S. 1990. Neighborhood effects on fruit removal by birds: a field experiment with *Viburnum dentatum* (Caprifoliaceae). *Ecology* **71**:1289-1298.
- Sargent, S. 1995. Seed fate in a tropical mistletoe: The importance of host twig size. *Functional Ecology* **9**:197-204.
- Schlamowitz, R., F. R. Hainsworth, and L. L. Wolf. 1976. On the Tongues of Sunbirds. *The Condor* **78**:104-107.
- Schoen, D. J., M. T. Morgan, and T. Bataillon. 1996. How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**:1281-1290.
- Schupp, E. W. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. *Vegetatio* **107-108**:15-29.

- Sekercioglu, C. H., G. C. Daily, and P. R. Ehrlich. 2004. Ecosystem consequences of bird declines. *Proceedings of the National Academy of Sciences of the United States of America* **101**:18042-18047.
- Smart, C. 1952. The life history of *Tupeia Cham. et Sche.* *Transactions of the Royal Society of New Zealand* **79**:459-466.
- Snow, A., T. Spira, R. Simpson, and R. Klips. 1996. The ecology of geitonogamous pollination. Pages 191-216 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral Biology: Studies on Floral Evolution in Animal-pollinated Plants*. Chapman and Hall, New York.
- Snow, B. K. and D. W. Snow. 1971. The feeding ecology of tanagers and honeycreepers in Trinidad. *Auk* **88**:291-322.
- Snow, D. 1971. Evolutionary aspects of fruit eating by birds. *Ibis* **113**:194-202.
- Sobrevila, C. and M. T. K. Arroyo. 1982. Breeding Systems in a Montane Tropical Cloud Forest in Venezuela. *Plant Systematics and Evolution* **140**:19-37.
- Soto-Gamboa, M. and F. Bozinovic. 2002. Fruit-disperser interaction in a mistletoe-bird system: A comparison of two mechanisms of fruits processing on seed germination. *Plant Ecology* **159**:171-174.
- Stanton, M. L. 2003. Interacting Guilds: Moving beyond the Pairwise Perspective on Mutualisms. *American Naturalist* **162**.
- Stebbins, G. L. 1950. *Variation and evolution in plants*. Columbia University Press, New York.
- Stiles, F. G. 1978. Ecological and Evolutionary Implications of Bird Pollination. *American Zoologist* **18**:715-727.
- Stiles, F. G., and C. E. Freeman. 1993. Patterns in Floral Nectar Characteristics of Some Bird-Visited Plant Species From Costa Rica. *Biotropica* **25**:191-205.
- Stratton, D. A. 1989. Longevity of individual flowers in a Costa Rican cloud forest: ecological correlates and phylogenetic constraints. *Biotropica* **21**:308-318.
- Sun, C., A. R. Ives, H. J. Kraeuter, and T. C. Moermond. 1997. Effectiveness of three turacos as seed dispersers in a tropical montane forest. *Oecologia* **112**:94-103.
- Swynnerton. 1916. Short cuts by birds to nectaries. *Journal of the Linnean Society of Botany* **43**:381-415.
- Tadey, M. and M. A. Aizen. 2001. Why do flowers of a hummingbird-pollinated mistletoe face down? *Functional Ecology* **15**:782-790.
- Thompson, J. N. 1994. *The coevolutionary process*. The University of Chicago Press, Chicago.
- Thompson, J. N. 2005. *The geographic mosaic of coevolution*. The University of Chicago Press, Chicago.

- Traveset, A. and M. Verdu. 2002. A meta-analysis of the effect of gut treatment on seed germination. *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation*:339-350.
- Traveset, A. and M. F. Willson. 1998. Ecology of the fruit-colour polymorphism in *Rubus spectabilis*. *Evolutionary Ecology* **12**:331.
- Tsujita, K., S. Sakai, and K. Kikuzawa. 2008. Does individual variation in fruit profitability override color differences in avian choice of red or white *Ilex serrata* fruits? *Ecological Research* **23**:445.
- Vaknin, Y., Y. Yom Tov, and D. Eisikowitch. 1996. Flowering seasonality and flower characteristics of *Loranthus acaciae* zucc. (Loranthaceae): Implications for advertisement and bird-pollination. *Sexual Plant Reproduction* **9**:279-285.
- Van der Pijl, L. 1961. Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* **15**:403-416.
- Van Tieghem, P. E. L. 1895. Sur le groupement des especes en genres dans les Loranthacees a calice gamosepale et antheres basifixes ou Dendrophthoees. *Bulletin de la Societe Botanique de France* **42**:241-272.
- Vidal-Russell, R. and D. L. Nickrent. 2008a. Evolutionary relationships in the showy mistletoe family (Loranthaceae). *American Journal of Botany* **95**:1015-1029.
- Vidal-Russell, R. and D. L. Nickrent. 2008b. The first mistletoes: Origins of aerial parasitism in Santalales. *Molecular Phylogenetics and Evolution* **47**:523-537.
- Walker, B. H. 1992. Biodiversity and ecological redundancy. *Conservation Biology* **6**:18-23.
- Wang, B. C. and T. B. Smith. 2002. Closing the seed dispersal loop. *Trends in Ecology and Evolution* **17**:379-385.
- Waser, N. M., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in Pollination Systems, and Why it Matters. *Ecology* **77**:1043-1060.
- Watson, D. M. 1997. The importance of mistletoe to the white-fronted honeyeater *Phylidonyris albifrons* in western Victoria. *Emu* **97**:174-177.
- Watson, D. M. 2001. Mistletoe - A keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics* **32**:219-249.
- Weins, D. and B. A. Barlow. 1971. The cytogeography and relationships of the Viscaceous and Eremolepidaceous mistletoes. *Taxon* **20**:313-332.
- Wenny, D. G. 2001. Advantages of seed dispersal: A re-evaluation of directed dispersal. *Evolutionary Ecology Research* **3**:51-74.
- Wenny, D. G. and D. J. Levey. 1998. Directed seed dispersal by bellbirds in a tropical cloud forest. *Proceedings of the National Academy of Sciences of the United States of America* **95**:6204-6207.

- Wheelwright, N. T. 1985a. Competition for dispersers, and the timing of flowering and fruiting in a guild of tropical trees. *Oikos* **44**:465-477.
- Wheelwright, N. T. 1985b. Fruit-Size, Gape Width, and the Diets of Fruit-Eating Birds. *Ecology* **66**:808-818.
- Wheelwright, N. T. and G. H. Orians. 1982. Seed Dispersal by Animals: Contrasts with Pollen Dispersal, Problems of Terminology, and Constraints on Coevolution. *The American Naturalist* **119**:402-413.
- White, F. 1978. The Afromontane Region. Pages 463-513 in M. J. A. Werger and A. C. Van Bruggen, editors. *Biogeography and ecology of Southern Africa*. Dr W Junk, The Hague.
- White, F. 1981. The history of the Afromontane archipelago and the scientific need for its conservation. *African Journal of Ecology* **19**:33-54.
- White, F. 1983. *The Vegetation of Africa*. UNESCO, Paris.
- Whitney, K. D. and C. E. Lister. 2004. Fruit colour polymorphism in *Acacia ligulata*: Seed and seedling performance, clinal patterns, and chemical variation. *Evolutionary Ecology* **18**:165.
- Willson, M. F., H. J. Michaels, R. I. Bertin, B. Benner, S. Rice, T. D. Lee, and A. P. Hartgerink. 1990. Intraspecific Variation in Seed Packaging. *American Midland Naturalist* **123**:179-185.
- Willson, M. F. and C. J. Whelan. 1990. The Evolution of Fruit Color in Fleshy-Fruited Plants. *The American Naturalist* **136**:790-809.
- Willson, M. F. and C. J. Whelan. 1993. Variation of dispersal phenology in a bird-dispersed shrub, *Cornus drummondii*. *Ecological Monographs* **63**:151-172.
- Wolf, L. L., G. Stiles, and F. R. Hainsworth. 1976. Ecological organisation of a tropical highland hummingbird community. *Journal of Animal Ecology* **45**.
- Wrangham, R. W., C. A. Chapman, and L. J. Chapman. 1994. Seed dispersal by forest chimpanzees in Uganda. *Journal of Tropical Ecology* **10**:355-368.
- Yan, Z. 1993. Seed Dispersal of *Amyema preissii* and *Lysiana exocarpi* by Mistletoebirds and Spiny-cheeked Honeyeaters. *Emu* **93**.

Appendix 1

Table 1: Results from the GLM ANOVA model comparing *G. braunii* flower opening under bagged, caged and natural pollination treatments across twelve plants (n=535 flowers) in 2007/08 and 2008/09. A binomial error distribution with a chi-squared test for significance was used.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			56	240.45	
Plant	12	55.974	44	184.48	P < 0.001
Year	1	31.171	43	153.31	P < 0.001
Treatment	2	79.437	41	73.87	P < 0.001
Year:Treatment	2	3.498	39	70.37	0.174

Table 2: Results from the binomial GLM ANOVA model using Chi squared analysis to compare the number of open *G. braunii* flowers which reached stage one versus stage two pollination under bagged, caged and natural pollination treatments across twelve plants (n=408 flowers) in 2007/08 and 2008/09.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			55	128.232	
Plant	12	26.227	43	102.004	0.010
Year	1	21.542	42	80.463	P < 0.001
Treatment	2	34.382	40	46.080	P < 0.001
Year:Treatment	2	4.747	38	41.333	0.093

Table 3: Results from the GLM ANOVA model comparing *G. braunii* fruit set versus failure under bagged, caged and natural pollination treatments across thirteen plants (n=750 flowers) in 2007/08 and 2008/09. A binomial error distribution was used with a chi-squared test for significance.

Factor	Df	Deviance	Residual Df	Residual Deviance	P(Chi)
Null			74	386.52	
Plant	12	40.63	62	345.89	P < 0.001
Year	1	70.84	61	275.05	P < 0.001
Treatment	4	200.44	57	74.61	P < 0.001
Year:Treatment	2	3.25	55	71.36	0.20

Appendix 2

Hard round flower galls approximately 1 cm in diameter, were very common on *G. braunii* plants and were initially mistaken for fruits due to their frequency. Ants were occasionally observed crawling over these galls and appeared to be ‘cleaning’ the galls, though their exact behaviour was unclear (Figure 1).



Figure 1: Ants on *Globimetula braunii* flower galls